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14. ABSTRACT PTEN is lost in a significant percentage of prostate cancers, which has been verified in animal models. However, these models fail to assess the role of PTEN in development given the usage of promoters regulated at differentiation. Results generated by knocking down PTEN in a human model of normal prostatic regeneration show both an arrested differentiation identified with progenitor cell markers and transdifferentiation to various urogenital organs including bladder, urethra and intestine. Each organ type is surrounded by representative differentiated stroma suggesting that gradients of PTEN expression may dictate the development of various urogenital organs. Stromal changes have also been associated with disease progression, which is difficult to model in transgenic animals due to the lack of appropriate promoters. To overcome this limitation, a human prostate fibroblast cell line has been generated along with a number of derivatives ectopically expressing chemokines and cytokines of					
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Introduction

Benign prostatic hyperplasia (BPH) is an enlargement of the transitional and periurethral area of the prostate gland that affects 60% of men over 60 years old. 50% of men at age 50 have lower urinary tract symptoms (LUTS) arising from BPH and 20-30% require surgical intervention by age 80. The etiology of BPH is still unknown although analyses of comorbidities and retrospective studies of patients on medications for comorbidities have revealed insights into potential molecular mechanisms. The current treatment regimen for BPH is initially to place patients on uroselective (alfuzosin SR, tamsulosin) or nonuroselective (doxazosin, terazosin) α_1 -adrenergic blockers to relax smooth muscle contraction and relieve urinary flow symptoms. This provides almost immediate relief. When or if this treatment fails (since it does not treat prostate volume), 5 α -reductase inhibitors (dutasteride, finasteride) are employed, which, after 6 months of treatment, slowly lower prostatic volume by inhibiting enzymatic production of androgen, thereby reducing LUTS.

Prostate cancer (CaP) is the most common non-skin malignancy and second most common cause of cancer deaths in American men. Cancer of the prostate is screened serologically for prostate specific antigen (PSA). While PSA screening in the U.S. has clearly coincided with stage migration toward lower risk disease at diagnosis, the proof that such screening improves survival has been more controversial (Andriole, 2009; Schroder, Roobol et al., 2009; Shapley, Kenfield et al., 2009). Complicating this concern is the observation that prostate cancer is being over-treated in a substantial number of men by interventions such as surgery or radiation therapy, which have significant associated morbidities. Active surveillance may be an appropriate alternative for some men, but can prove unnerving for both patients and clinicians. The predominant medical intervention is androgen deprivation therapy (ADT), but this is an inappropriate alternative for localized disease in most men due to its substantial associated long-term morbidity. Given the success of statins at lowering death related to heart disease, men are now more than ever exposed by advancing age to the onset of prostatic disease. *Therefore, novel dietary or chemopreventative agents that could be utilized in managing benign disease and in preventing lethal transformation are urgently needed.*

Our lab has previously shown that the human prostate cancer microenvironment can promote disease progression via subtle changes in the production of soluble factors (Olumi, Grossfeld et al., 1999; Ao, Williams et al., 2006; Ao, Franco et al., 2007). Targeting therapies to the tumor stroma are attractive for a number of reasons including greater genetic stability of the cells comprising the microenvironment and the ability to target multiple cell types and pathways to potentially elicit synergistic effects. To target these interactions, however, a better understanding of the paracrine interaction among fluctuating populations of stroma and the molecular signaling that regulates glandular and stromal differentiation is needed. A genetic analysis of prostate tumors in human patients has revealed a number of statistically significant mutations in the PI3K/AKT/PTEN pathways, which have been modeled in genetically modified mice. Notably for this proposal, mutations to PTEN occur in approximately 30% of primary tumors and 60% of metastatic tumors (Suzuki, Freije et al., 1998), making PTEN one of the most highly mutated or lost genes in prostate cancer. Mouse models of PTEN inactivation demonstrate a susceptibility to tumorigenicity compounded by inactivations of other tumor suppressor genes like Nkx3.1 (Lei, Jiao et al., 2006), p53 (Chen, Trotman et al., 2005), and p27 (Di Cristofano, De Acetis et al., 2001).

In addition to the identification of consistent epithelial mutations correlated with and experimentally verified as important for prostate cancer initiation, genetic and morpho/immuno-profiling of tumor microenvironments in breast (Finak, Sadekova et al., 2006; Finak, Bertos et al., 2008) and prostate (Ayala, Tuxhorn et al., 2003) have revealed a potentially key role for stromal chemokines in tumor progression. Unfortunately, modeling the stromal microenvironment is difficult to control and significant differences between mouse and human stromal compartments only compound the difficulty. To this end, **the objective of this proposal is to determine whether the activation of the stromal microenvironment, as defined by chemokine expression, promotes the progression of PTEN-deficient prostate cancer epithelia.** The rationale for performing the proposed work is that, while we know that the cancer stromal phenotype correlates well with patient prognosis, the consequences resulting from specific genetic lesions and the manner in which these are linked to stromal changes are currently not understood. This will allow us to identify and screen specific pathways for therapeutic intervention. To accomplish this goal, we will use newly characterized and

reported normal human prostate epithelial cell lines in a modular, *in vivo*, model of stromal heterogeneity to answer specific questions regarding the role of the tumor microenvironment in the stepwise progression of tumorigenesis.

Body

The **hypothesis** is that morphologic changes due to increased AKT signaling are: 1) associated with carcinogenesis in the prostatic epithelium, and 2) result in phenotypic and functional changes in adjacent stromal cells that sustain a vicious cycle of microenvironmentally-driven tumor progression. The precedent set by transgenic animals with aberrant PI3K/AKT/PTEN signaling suggests additional cooperative mutations in cell cycle pathways are necessary to drive carcinogenesis. In addition, specific growth signaling (mTor) pathways have been shown to mediate the proliferative phenotype induced by Akt activation (Blando, Portis et al., 2009). Unfortunately, determining whether specific stromal genotypes (or naturally occurring subpopulations) contribute to this process using transgenic animals is hindered by the lack of appropriate stromal promoters (Jackson, Franco et al., 2008). Accordingly, as reviewed by the author and collaborators (Franco, Shaw et al., 2010; Strand, Franco et al., 2010; Strand and Hayward, 2010), new models of stromal-epithelial interactions are being developed in our group to empirically determine the contribution of specific stromal sub-populations or chemokines to tumor progression (Franco, Jiang et al., 2011; Kiskowski, Jackson et al., 2011). Notably, in addition to tumor cell signaling pathways, chemokines, cytokines and their cognate receptors that are active in the tumor microenvironment are attractive targets for therapeutic intervention. We have shown that altered stromal gene expression profiles can elicit tumor progression of SV40 Large T-antigen-initiated epithelia (Ao, Franco et al., 2007); however, the identity of specific genetic lesions that initiate a reactive microenvironment are unknown. Our newly-available, functionally normal human prostate epithelial cell lines (Jiang, Strand et al., 2010) are a valuable tool for linking the histological effects of specific epithelial lesions on the tumor microenvironment. In addition, the identity of genetic lesions that make a tumor cell more susceptible to specific types of tumor microenvironments are unknown and will be addressed using an optimized *in vivo* model of tumor development incorporating an isogenic series of human prostatic epithelial cells.

The original stated goals in the approved Statement of Work for Task 1 included generating normal (BHPRe1) and initiated (BPH1) human prostate epithelia with stably transfected, tetracycline-inducible PTEN shRNA for assessment of proliferation and differentiation *in vitro* and for *in vivo* xenografting in combination with rat urogenital mesenchyme into the kidney capsule of SCID mice. Tissue recombination xenografts were generated in SCID mice and assessed histologically for morphology, epithelial and stromal differentiation, inflammatory cell recruitment, invasion, proliferation and apoptosis. Unfortunately, no significant phenotype was observed in tetracycline-treated animals. After further molecular analysis *in vitro*, it was noted that the treatment of cells with tetracycline resulted in the upregulation of PPARgamma, which is thought to antagonize the pro-proliferative effect of activated AKT thereby negating the effect of PTEN suppression.

There has been a wealth of data recently that have implicated defective lipid metabolism in prostate disease. After encountering nothing particularly new about PTEN loss in our human cells (expansion of basal cells similar to the mouse PTEN knockout model), it was decided that following the trail of data indicating that PPARgamma signaling may be antagonizing AKT signaling may be worth pursuing. In fact, given the inability of human prostate CAFs to induce tumorigenesis in PTEN-deficient epithelia *in vivo*, the following data will describe a mouse model of PPARgamma and PTEN function in epithelia, which replaced the stromal focus in Aim 2.

PPARgamma is a nutritionally regulated nuclear transcription factor that regulates the expression of a number of metabolic enzymes in mitochondria and peroxisomes. PPARgamma is a target for insulin insensitivity in diabetes using a series of drugs called thiazolidinediones (TZDs) that seem to increase insulin sensitivity by decreasing lipotoxicity in a variety of cell types. Importantly, type II diabetes is now recognized as a comorbidity for benign prostate disease and prostate cancer mortality (Jiang, Strand et al., 2011); however, the mechanisms by which TZDs alleviate insulin insensitivity are poorly understood (Sears, Hsiao et al., 2009). The prostate is an insulin target tissue and insulin signals

through the AKT pathway. Therefore, it is essential to unravel the etiological implications of synergism and crosstalk between the AKT and PPARgamma signaling pathways. By generating cell models of PPARgamma and PTEN deficiency, we have gained valuable insights into the role of insulin signaling and lipid metabolism in prostate differentiation and disease. These goals have now been incorporated into Aim 1. The hypothesis has therefore adjusted to include this new understanding that systemic metabolic diseases affect prostate disease and that AKT activated lipogenesis must be understood within the broader context of the lipogenic/lipolytic balance controlled by nutritionally regulated genes like PPARgamma (Menendez, 2010).

The AKT pathway activates *de novo* lipogenesis through fatty acid synthase and acetyl coa carboxylase, which take acetyl coa from the TCA cycle in mitochondria and use it for the construction of complex lipids. Normally, *de novo* lipogenesis occurs only during development, but is reactivated in cancer (Flavin, Zadra et al., 2011). Understanding the etiology of this process of metabolic rearrangement is important to the management of prostate differentiation and overall health, which could possibly prevent or delay tumor initiation. PPARgamma is well-known to induce adipocyte differentiation, but its function in prostate is poorly understood. Given its role in lipid and glucose metabolism, it was decided to further manipulate the PTEN knockdown cells by knocking down PPARgamma as well. Similar to the original focus of Aim 2, the hypothesis was that the expansion of basal cells phenotype (and not adenocarcinoma) due to AKT activation in human prostate regeneration experiments might progress towards malignancy if the 'brakes' were removed.

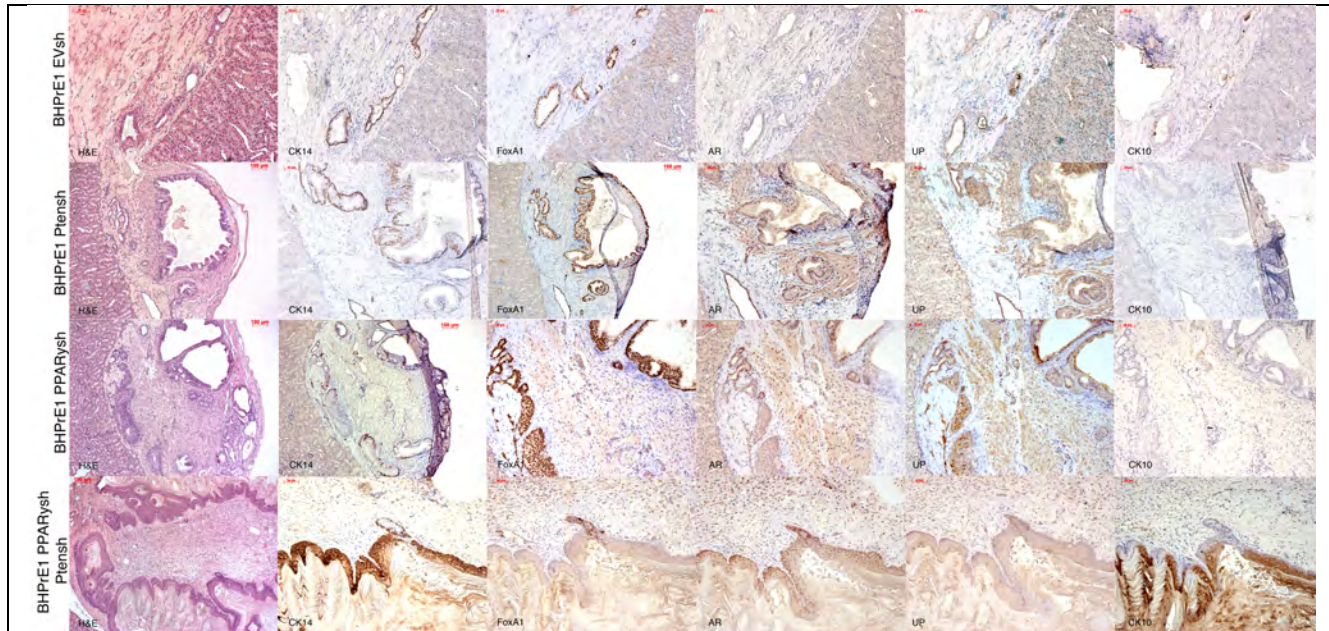


Figure 1. Human prostate epithelial intermediate cells regenerate urothelial tissue in combination with bladder mesenchyme in PPARg-deficient cells and regenerate squamous metaplasia in PPARg/PTEN-deficient cells due to a loss of FoxA1 expression. CK14 (basal marker), FoxA1 (urogenital marker), AR (prostate marker), UP (urothelial marker), CK10 (squamous marker).

Interestingly, it was discovered that the majority of PPARg-deficient tissue recombinants led to the regeneration of urothelial tissue, even with prostatic mesenchyme. In fact, qPCR for uroplakin 2, a marker of differentiated urothelial tissue was 20-fold higher in PPARgamma knockdown cells (data not shown). Figure 1 shows a panel of markers in tissue recombination xenografts made with PPARg, PTEN or PPARgamma/PTEN knockdown derivatives and bladder mesenchyme. The parental cells do not regenerate bladder tissue. The most interesting phenotype was the squamous metaplasia regenerated by the PPARg1/2 and PTEN double knockdown cells, which is thought to be a precursor to bladder carcinogenesis (Ahmad, Barnettson et al., 2008). A model for squamous metaplasia of the bladder does not exist to date, neither does a normal human bladder cell line. A molecular mechanism linking FoxA1 loss to squamous metaplasia has been characterized in both our human tissue regeneration model (row 3) and in resected human bladder tissue (data not shown). It is difficult to

determine whether PPAR γ is regulating FoxA1 levels in our cells since they are progenitor in culture and therefore do not express FoxA1 until recombined with inductive mesenchyme *in vivo*. Previous studies have, however, linked PPAR γ to FoxA1 expression (Varley, Bacon et al., 2009) and previous work has also implicated the AKT pathway (implicitly via EGFR mutations) with the development of squamous metaplasia of the bladder (Guo, Fine et al., 2006). The conclusions based on these results are that high PPAR γ levels are necessary for specification of prostate differentiation from these human urogenital progenitor cell lines and that the mesenchyme of fetal prostate probably acts on this lipid metabolic axis to induce differentiation. A manuscript describing these observations in our human cell lines is currently underway.

To examine the interaction between PPAR γ and PTEN in a cleaner knockout system (vs. shRNA in the human cell lines), we generated a prostate epithelial cell line from PBCre4;PPAR γ flox/flox mice (mPrE^{PPAR γ KO}), which were published in our lab as having an autophagic phenotype (Jiang, Fernandez et al., 2010). We then rescued mPrE^{PPAR γ KO} with either PPAR γ 1 or PPAR γ 2 to determine their individual function. Microarray analysis revealed that the majority of genes regulated by PPAR γ are enzymes involved in steroid biosynthesis (CYP proteins) and lipid processing (lipoprotein lipase), which were confirmed by qPCR in Figure 2. Interestingly, androgen receptor expression is coordinately linked to PPAR γ restoration (Figure 2), which suggests a positive role for mitochondrial

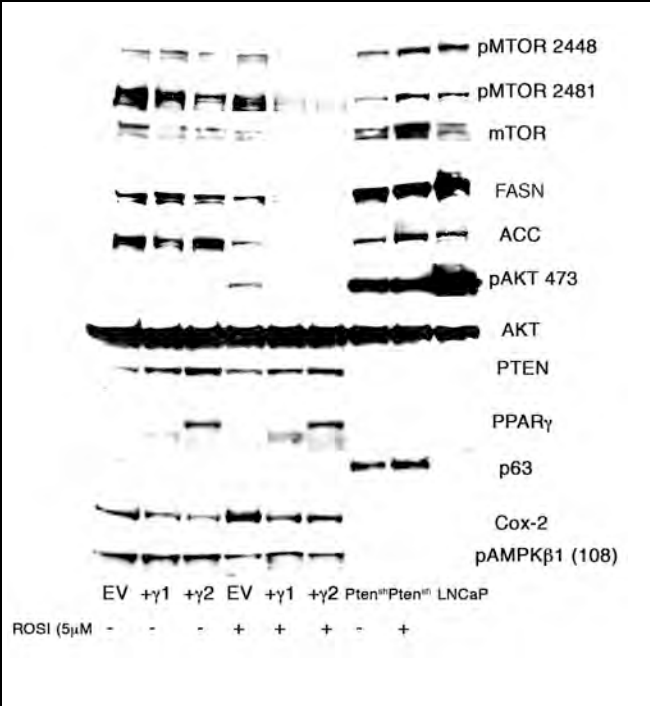


Figure 3. PPAR γ restoration and agonization reduces lipogenesis and protein synthesis.

PTEN knockdown cells and the PTEN knockout mouse prostate model, p63 is increased (other basal cell markers like ck5/14 are also increased). When these cells were recombined with inductive fetal

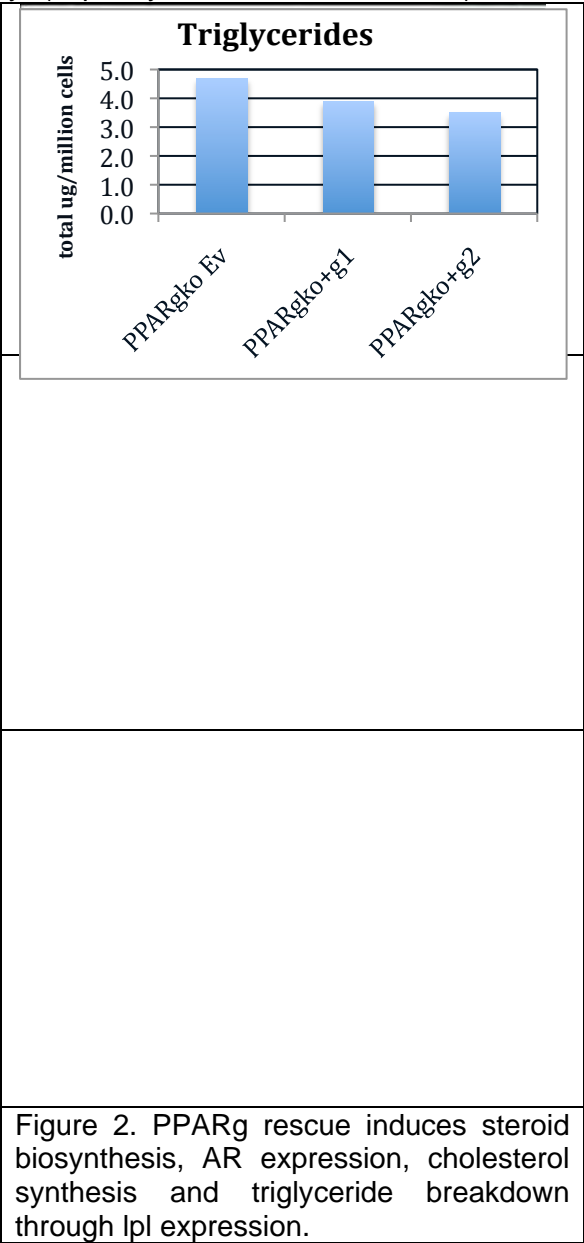


Figure 2. PPAR γ rescue induces steroid biosynthesis, AR expression, cholesterol synthesis and triglyceride breakdown through lpl expression.

metabolism of lipids in prostatic differentiation. As shown by Western blot in Figure 3, treatment of the control and rescue cells with a PPAR γ agonist (rosiglitazone) resulted in the inhibition of mTOR signaling and *de novo* lipogenesis (ACC, FASN), an increase in PTEN levels, and a decrease in oxidative stress (COX-2). However, downregulation of PTEN by shRNA in the mPrE^{PPAR γ KO} cells resulted in the activation of AKT and its downstream mediator mTOR as well as increasing *de novo* lipogenesis through FASN and ACC activation. In addition, as noted in our human

UGM in kidney capsule xenografts, it was found that the activation of AKT in these cells resulted in a pronounced basal phenotype, as shown in Figure 4. The mPrE^{PPARgKO} cells alone regenerate characteristic adenocarcinoma, with a majority of acini displaying a luminal phenotype, with no basal cells.

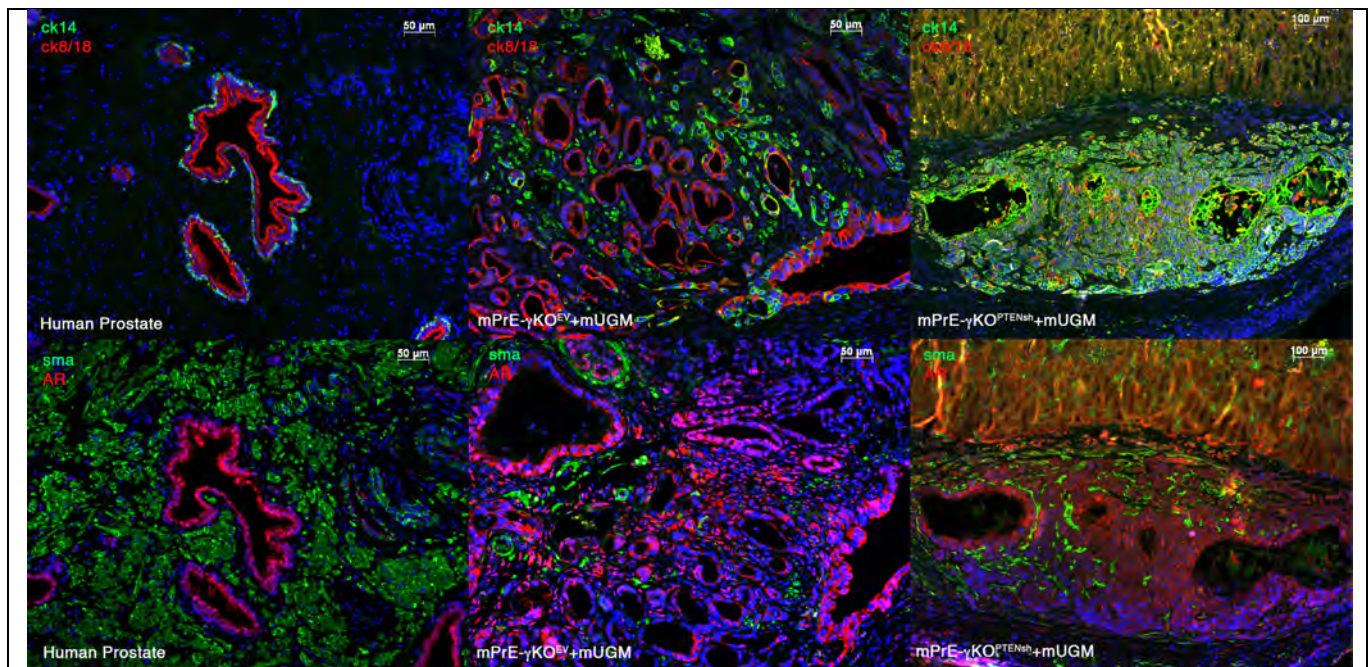


Figure 4. PPARg deficiency results in a failure to maintain an acinar bilayer (basal/luminal populations) resembling the luminal phenotype of adenocarcinoma while PTEN loss drives a total basal phenotype with low androgen receptor expression.

Because of the link between diet, obesity, and dyslipidemia in prostate disease, thin layer chromatography was performed on the mPrE^{PPARgKO} and rescue cells to determine the fatty acid content of various lipid species. Interestingly, PPARg2 seems to predominantly regulate the long chain fatty acid content of phospholipids in addition to increasing their total levels (data not shown). Correlated with the increased levels of cholesterol biosynthetic enzymes, there was also a significant increase in cholesterol levels. Both of these lipid species are intrinsic to secretory cell types.

In summary, the links between nutrient metabolism and prostatic differentiation are now being unraveled in the context of PPARgamma and AKT signaling. Further work on the molecular interaction between PPARgamma and AKT will be performed and a manuscript describing these results is being drafted.

Task 1. Determine the consequence of PTEN suppression on glandular and stromal differentiation of human prostate (months 1-12):

1. Develop the PTEN shRNA expression constructs for stable expression (month 1). **Completed**
1. Develop and characterize the BPH1 and BHPRE1 stable cell lines with PTEN shRNA expression (month 2). **Completed**
 - a. Determine expression of PTEN by western blot
 - b. Quantification of proliferation by [³H]-thymidine incorporation and cell cycle FACS
 - c. Quantification of apoptosis by caspase-3 western blot and ICC and cell cycle FACS
 - d. Determine expression of E-cadherin, p53 and phospho-Akt by western blot and ICC.
3. Tissue recombination xenografting of stable epithelial cell lines with rUGM for 3, 6, 9 month periods with or without tetracycline. Two mice per cell line per time point are required (months 2-11). **Completed with modifications described below.**

4. Immunohistochemical analysis of epithelial and stromal compartments (month 12).

Completed IHC phase

- a. Evaluation of tissue differentiation by IHC
- b. Evaluation of inflammatory cell recruitment by IHC
- c. Quantification of proliferation and apoptosis by IHC
- d. Statistical analysis and manuscript preparation.

In order to study the effect of specific stromal subpopulations on normal and PTEN-deficient prostate morphology, prostate stromal cell lines overexpressing specific chemokines were generated. First, coincident with the production of the BHPRE1 prostate epithelial cell line, a prostate stromal cell line was immortalized by hTERT called BHPRS. Although stable expression of the tetracycline inducible p53 shRNA in stroma as described in the original statement of work was performed, due to technical limitations of the tetracycline system described above and the emergence of more influential stromal chemokines, work was adjusted to make the stromal cell lines that overexpress the chemokines TGF β , SDF1 α or IL-8, which are all commonly overexpressed in prostate tumor stroma. ELISA for the TGF β and SDF1 α cell lines. Because these constructs also express GFP, these cells can be tracked *in vivo* when recombined with epithelia and rUGM using immunohistochemistry. An enormous amount of optimization went into determining the technical limits of this type of model. For instance, at high levels, GFP is toxic so cells had to be resorted (FACS) for lower expression. In addition, the ratio of UGM to BHPRS cells had to be optimized in order to maintain proper tissue regeneration and differentiation. An assessment of the phenotypic effect of these chemokines on glandular differentiation was performed over the last year. Unfortunately, the regeneration of these grafts was inconclusive and the second task was shelved. Instead, the metabolic effect of cooperative loss of PPARgamma with PTEN loss in epithelia was focused upon as described above.

Status of progress in relation to the original SOW is summarized below:

Task 2. Determine the consequence of CAFs or NPFs with decreased p53 on human epithelia with decreased PTEN (months 12-24):

1. Develop the p53 shRNA expression construct for stable expression (month 12).
Completed, also generated other stromal cell lines as described
2. Develop and characterize the NPF stable cell line with p53 shRNA expression (month 12-14). **Ongoing with modifications**
 - a. Determine expression of p53 by western blot
 - b. Quantification of proliferation by [³H]-thymidine incorporation and cell cycle FACS
 - c. Determine expression of p21, p27 and cyclin D1 by western blot and ICC.
 - d. Determine paracrine effect of NPF-p53sh cells on neighboring fibroblasts by conditioned media experiments followed by western blot, ICC in cell culture and IHC in xenografts.
3. Tissue recombination xenografting of stable epithelial cell lines with CAF or NPF-p53sh for 3, 6, 9 month periods with or with tetracycline. Two mice per cell line per time point are required. (months 14-23). **Inconclusive**
4. Immunohistochemical analysis of epithelial and stromal compartments (month 24).
Inconclusive
 - a. Evaluation of tissue differentiation by IHC
 - b. Evaluation of inflammatory cell recruitment by IHC
 - c. Quantification of proliferation and apoptosis by IHC
 - d. Statistical analysis.

Key research accomplishments

- Epithelial cell lines were generated and characterized in vitro.
- The BHPRE1 human prostate intermediate cell line (ref 2) appears to be a basal-like cell capable of differentiation to multiple endodermal derivatives of the urogenital sinus depending on the status of PPARg expression (fig 1)

- PPAR γ /PTEN derivatives were generated and characterized *in vitro* and *in vivo*.
- A mechanistic understanding of urothelial and prostatic differentiation is being linked to the regulation of nutrient metabolism by PPAR γ and PTEN.

Reportable outcomes

The following publications were referenced by this training grant during the last year:

1. **Strand DW** and Hayward SW. Modeling stromal-epithelial interactions in disease progression. *Discov Med*. 2010 Jun;9(49):504-11.
2. David Barron*, **Douglas Strand***, Steven J. Ressler, Truong D. Dang, Gustavo E. Ayala, Michael Ittmann, and David R. Rowley. TGF- β 1 Induces an Age-Dependent Inflammation of Nerve Ganglia and Fibroplasia in the Prostate Gland Stroma of a Novel Transgenic Mouse. 2010 PLoS ONE 5(10): e13751. (* denotes equal authorship)
3. Omar E Franco, Ming Jiang, **Douglas W Strand**, James Peacock, Roger S Jackson 2nd, Patricia Revelo, Neil A Bhowmick, Simon W Hayward. Altered TGF- β Signaling in a Sub-population of Stromal Cells Promotes Prostate Carcinogenesis. *Cancer Research* 2011. Feb 15;71(4):1272-81.
4. Jiang M, Min YF, DeBusk L, Fernandez S, **Strand DW**, Hayward SW and Lin PC. Spontaneous immortalization of human dermal microvascular endothelial cells. *World J Stem Cells* 2010. October 26; 2(5): 114-120.
5. Ming Jiang, **Douglas W. Strand**, Omar E. Franco, Peter E. Clark, Simon W. Hayward. PPAR γ : A molecular link between systemic metabolic disease and benign prostate hyperplasia. *Differentiation* (2011),doi:10.1016/j.diff.2011.05.008

Conclusions

Significant progress has been made towards achieving the stated goals even though technical limitations were encountered. A number of publications relating to the stated goals have been published as listed in the reportable outcomes. Given the technical limitations with the human cells in tissue recombination, the original goals of Aim 2 were replaced with a more detailed look at the effect of PPAR γ and Pten on lipid metabolism and prostate differentiation. A manuscript is in preparation now entitled, "PPAR γ regulates prostate differentiation through inhibition of AKT-induced de novo lipogenesis".

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Invited Review

PPAR γ : A molecular link between systemic metabolic disease and benign prostate hyperplasiaMing Jiang^{a,**}, Douglas W. Strand^a, Omar E. Franco^a, Peter E. Clark^a, Simon W. Hayward^{a,b,*}^a Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN, USA^b Department of Cancer Biology, Vanderbilt-Ingram Cancer Center, Nashville, TN, USA

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ABSTRACT

The emergent epidemic of metabolic syndrome and its complex list of sequelae mandate a more thorough understanding of benign prostatic hyperplasia and lower urinary tract symptoms (BPH/LUTS) in the context of systemic metabolic disease. Here we discuss the nature and origins of BPH, examine its role as a component of LUTS and review retrospective clinical studies that have drawn associations between BPH/LUTS and type II diabetes, inflammation and dyslipidemia. PPAR γ signaling, which sits at the nexus of systemic metabolic disease and BPH/LUTS through its regulation of inflammation and insulin resistance, is proposed as a candidate for molecular manipulation in regard to BPH/LUTS. Finally, we introduce new cell and animal models that are being used to study the consequences of obesity, diabetes and inflammation on benign prostatic growth.

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Abbreviations: 15-LOX-2, 15-lipoxygenase-2; 15dPGJ2, 15-deoxy- Δ 12,14-prostaglandin J2; 15 S-15-HETE, hydroxyeicosatetraenoic acid; AIS, androgen insensitivity syndrome; AP, anterior prostate; AP2, fatty acid binding protein; AR, androgen receptor; BMI, body mass index; BPH, benign prostate hyperplasia; CaP, carcinoma of the prostate; CAT, catalase; Cdk5, cyclin-dependent kinase 5; COX-2, cyclooxygenase-2; DBD, DNA-binding domain; DHA, docosahexaenoic acid; DHT, dihydrotestosterone; DM, diabetes mellitus; ERK, extracellular signal-regulated kinase; ER α , estrogen receptor- α ; GPx, glutathione peroxidase; HDAC3, histone deacetylase-3; HDL, high-density lipoprotein; HFD, high-fat diet; IFN γ , interferon gamma; IGF-1, insulin-like growth factor-1; IL, interleukin; iNOS, nitric oxide synthase; IPSS, International Prostate Symptom Score; LBD, ligand-binding domain; LDL, low-density lipoprotein; LUTS, lower urinary tract symptoms; MAPK, mitogen activated protein kinase; MEK, MAPK kinases; MRI, magnetic resonance imaging; NCoR, nuclear receptor corepressor; NOD, non-obese diabetic mouse; NSAID, nonsteroidal anti-inflammatory drugs; PCPT, Prostate Cancer Prevention Trial; PGC1, PPAR γ co-activator-1; PPAR γ , peroxisome proliferator-activated receptor gamma; PPRe, peroxisome proliferator response element; PSA, prostate specific antigen; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; RXRs, retinoid X receptors; SOD, superoxide dismutase; T/E2, testosterone/estradiol; TA, transit amplifying; TRUS, transrectal ultrasound; TZDs, thiazolidinediones; UGM, urogenital mesenchyme; UGS, urogenital sinus; VP, ventral prostate; WHR, waist to hip ratio

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1. Introduction

Benign prostatic hyperplasia (BPH) is a focal enlargement of the periurethral region of the prostate seen in most aging men, which results in symptoms requiring clinical intervention in approximately a third of men over the age of 60. BPH has been tied to a larger collection of symptoms including frequency of urination, urgency, urinary incontinence, waking up multiple times at night to void (nocturia), weakened urinary stream, straining to void and a sense of incomplete emptying of the bladder. These morbidities have been grouped together under the general descriptor—lower urinary tract symptoms (LUTS).

Co-morbidities commonly seen in patients with BPH/LUTS include obesity and type 2 diabetes. Common co-morbidities have been recognized for more than 40 years (Bourke and Griffin, 1966); however, their systematic investigation did not start in earnest until much more recently. The likelihood of a BPH patient also having diabetes is elevated, and the progression and severity of LUTS in diabetic patients is more severe compared to non-diabetic BPH patients (Burke et al., 2006; Michel et al., 2000). Obesity, as measured by waist to hip ratio, is also strongly correlated with the incidence and severity of BPH (Kristal et al., 2007).

Both diabetes and obesity result in complex, and in some cases, shared systemic changes. For example both diabetes and obesity alter sex steroid hormone metabolism, and both may be considered to be “pro-inflammatory” conditions releasing chemokines that may well contribute to prostatic growth (Jerde and Bushman, 2009). In addition, diabetic and obese patients see significant changes in metabolic pathways. In particular, alterations in the ability to utilize glucose in diabetic patients result in increases in insulin-like growth factor-1 (IGF-1) activity. Inflammation is a common observation in human BPH and it has been suggested that the risk of BPH progression and acute urinary retention is greater in men with prostatic inflammation (McConnell et al., 2003; McVary, 2007).

Within this complex milieu of systemic alterations, we suggest that cellular management of lipid metabolism and proliferation may become disrupted. Peroxisome proliferator receptor-activated gamma (PPAR γ) sits at a key balance point in the regulation of cellular metabolism, differentiation and inflammatory responses. Alterations in the activity of this nuclear receptor have potential roles in both BPH and prostate cancer (Jiang et al., 2010b). PPAR γ activity is amenable to therapeutic manipulation. Although problems are evident with existing agonists, we speculate that combinatorial approaches that include these pathways may represent a new approach to tackle BPH in the context of common co-morbidities. Furthermore, the identification of common molecular pathways disrupted at a systemic level will likely yield key insights into the molecular pathogenesis of BPH/LUTS.

Here we will briefly review the nature of benign prostatic enlargement in the context of common co-morbidities that contribute

to a chronic inflammatory environment and thus potentially to prostatic enlargement. The role of cellular stress and metabolism will be examined as potential areas for clinical intervention.

2. Anatomy and development of the prostate

In 1912 Lowsley (1912) published a classic description of the anatomy and development of the human prostate, starting a debate over the nomenclature used to describe the organ that continued into the 1980s. Lowsley's work was based on studies of fetal glands. He described a dorsal or posterior lobe, a median and two lateral lobes, and additionally a ventral lobe that atrophied after birth. In the adult human these lobes are fused and cannot be separated or defined by dissection, giving rise over the years to a number of different views on the anatomic division of the human prostate (Franks, 1954; Hutch and Rambo, 1970; McNeal, 1984; Tissell and Salander, 1984). The situation is further confused by the fact that in most other animals, including other primates, the various prostatic lobes are separable in varying degrees on anatomical, histological and physiological bases.

The nomenclature now most commonly used to describe the human prostate is that pioneered and developed by McNeal (1984). This divides the prostate into three major anatomically separate and histologically distinct areas as shown in Fig. 1. These are (1) the non-glandular fibromuscular stroma that surrounds the organ and (2) two glandular regions termed the peripheral and central zones. Both of these zones contain a complex but histologically distinct ductal system. In addition, McNeal also describes a third, smaller, glandular region surrounding the prostatic urethra known as the transition zone.

Following McNeal's description, the peripheral zone ducts exit directly laterally from the postero-lateral recesses of the urethral wall. The system is described as consisting of small, simple round to oval acinar structures emptying into long narrow ducts surrounded by a stroma of loosely arranged and randomly interwoven muscular bundles. Ducts and acini are lined with pseudostratified columnar epithelium. This area is the principal site of prostatitis and carcinoma of the prostate (CaP), although not of benign prostatic hyperplasia (BPH). Within the peripheral zone is included the proximal urethral segment of the prostate. This comprises the region of the prostate between the base of the urinary bladder and the verumontanum (the area where the ejaculatory ducts feed into the urethra). The principal feature of this region, which comprises around 5% of the total prostate mass, is the preprostatic sphincter. The sphincter is a cylindrical sleeve of smooth muscle that stretches from the base of the bladder to the verumontanum.

The central zone ducts, in McNeal's model, course proximally, closely following the ejaculatory ducts. These ducts and acini are described as much larger than those of the peripheral zone and of

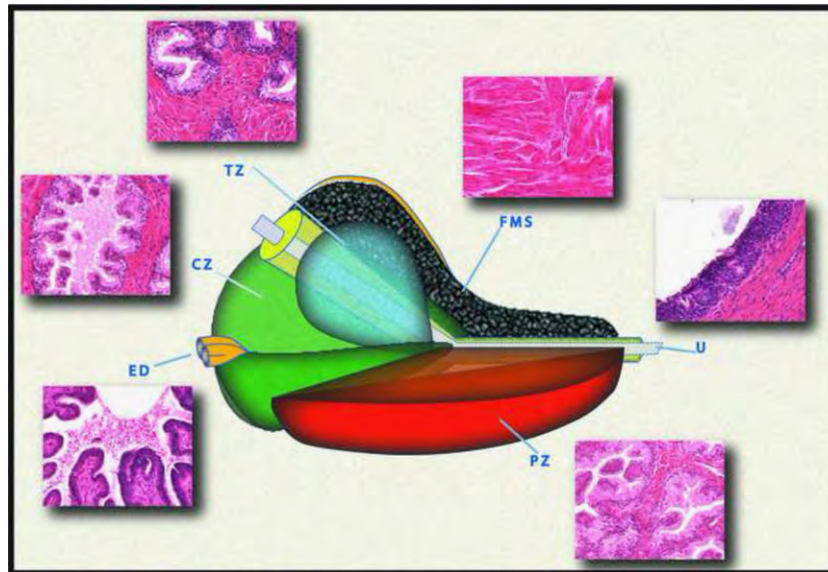


Fig. 1. Zonal anatomy of the human prostate, following McNeal. John McNeal pioneered a zonal anatomic subdivision of the prostate shown stylistically here. The central zone (CZ) surrounding the ejaculatory ducts (EDs), and a surrounding peripheral zone (PZ) surrounded by a fibromuscular stroma (FMS). The transition zone (TZ) surrounding the urethra below the bladder neck is the major location of benign prostatic hyperplasia and, due to its location, results in urethral compression.

irregular contour. The acini are polyhedral in cross section. The muscular stroma is much more compact than in the peripheral zone. Comparatively little disease is detected in the central zone, an observation that underlines the importance of a detailed knowledge of prostatic biology as a means of understanding the underlying susceptibility to morbidity.

The physiological links between the testis and the prostate have been known for many years. John Hunter, in 1786 in his "Observations on the glands situated between the rectum and the bladder, called vesiculae seminales" wrote, "the prostate and Cowper's glands and those of the urethra which in the perfect male are soft and bulky with a secretion salty to the taste, in the castrated animal are small, flabby, tough and ligamentous and have little secretion" (cited in Geller, 1989). The requirement for active androgenic participation in the formation of the prostate is illustrated by both human and non-human organisms with testicular feminization (tfm) also known as androgen insensitivity syndrome (AIS), caused by a congenital lack of functional androgen receptor (AR). Such organisms have a female phenotype and do not undergo prostatic development (Quigley et al., 1995).

The prostate is completely dependent for the maintenance of its structural and functional integrity on testicular androgens. In the human this is reflected in the natural history of the organ. The prostate is small in childhood, weighing around 2 g. At puberty it undergoes a phase of exponential growth, increasing in size to about 20 g. This corresponds to the rise in serum testosterone to adult levels. Mean prostatic weight then stabilizes and remains fairly constant until the end of the fifth decade of life. At this point mean prostatic weight (although not necessarily individual prostatic weight) begins to rise slowly. This rise reflects the incidence of BPH in the population (Walsh, 1984).

3. Stromal–epithelial interactions in prostatic development

Prostatic development begins with the formation of prostatic buds, which grow out from the fetal urogenital sinus (UGS). This occurs at about 10 weeks post-conception in the human (Kellokumpu-Lehtonen et al., 1980; Lowsley, 1912), around 19 days of gestation in the rat and 17 days in the mouse. Prostatic

development is a function of androgen action, and is dependent on the function of the fetal testis. Tissue recombination experiments have shown that testicular androgens act on AR in the mesenchymal cells of the urogenital sinus (UGM) (Cunha and Chung, 1981). Androgens elicit paracrine signals from the UGM that elicit epithelial budding, proliferation and differentiation into ductal structures (Cunha et al., 1987). Following their induction, solid prostatic epithelial cords grow into the surrounding mesenchyme, arborize, canalize and differentiate in a proximal-to-distal direction (from the urethra to the ductal tip) in the growing prostate. The epithelium proliferates and undergoes cytodifferentiation into luminal and basal subtypes (Hayward et al., 1996a, 1996b). Concurrently, the UGM proliferates and differentiates into prostatic smooth muscle and interfascicular fibroblasts (Hayward et al., 1996c). A number of paracrine signaling pathways have been shown to be active in the developing prostate (reviewed in (Marker et al., 2003; Thomson and Marker, 2006)). Recently, and of particular relevance to this review, a role for inflammatory cytokines in prostatic development has also been demonstrated (Jerde and Bushman, 2009).

4. Benign prostatic hyperplasia (BPH)

Human BPH originates immediately distal to the urinary bladder and surrounding the preprostatic sphincter. BPH nodules usually form as small structures within the transition zone (McNeal, 1984). These nodules occur within a clearly defined area either lateral or somewhat ventral to the urethral lumen. McNeal described this sharp focusing of BPH origin within the prostate into a region comprising about 2% of the total mass of the gland (McNeal, 1984). This pattern of genesis is reflected in prostates with larger more numerous BPH nodules (generally older age groups). In such cases, anatomic focusing is the same but not as restricted.

Nodules of BPH become increasingly common in the prostates of men with increasing age. Hyperplastic foci can be found starting around 40 years of age with rapidly increasing incidence to around 88% of men by the age of 80 years. Many of these people are asymptomatic; however BPH is the most common symptomatic benign neoplastic condition in humans (Kissane,

1989). BPH results in problems that include gross hematuria, bladder calculi, urinary retention and severe/bothersome LUTS.

Several different types of BPH nodules are recognized histologically. These include those with fibrous and/or muscular stroma, and these may or may not contain an epithelial component. Nodules in the periurethral area are often stromal in character with a few small glands penetrating from the periphery. Glandular nodules composed of ductal tissue are derived from newly formed small branches that bud from pre-existing ducts, grow into the adjacent stroma and repeatedly arborize to create a new architectural system within the nodule. These changes were described in classical studies by McNeal (1978, 1983, 1984, 1985).

Links between BPH and the testis are well established, based on the early observation that men castrated before the age of 40 years do not develop the condition. The Skoptzys, a Russian sect in which the males underwent ritual castration at 35 years, did not suffer from prostatic enlargement (Zuckerman, 1936). Furthermore it has been shown that the absence of testicular function from a young age, either from castration or hypopituitarism, prevents the occurrence of BPH in men living into the over 55 age group. Post-mortem examination of 28 such patients showed no histological evidence of BPH compared to an age matched control group where BPH was found in 50% of the patients (Moore, 1944).

During the 1970s, debate over the links between the prostate, the testis and BPH centered upon the steroid hormones involved and on the cellular subtype targeted. A number of reports over this period suggested that levels of dihydrotestosterone (DHT), the most biologically active androgen, were higher in BPH tissue than in normal prostate (Geller et al., 1976; Hammond, 1978; Krieg et al., 1979; Meikle et al., 1978; Siiteri and Wilson, 1970). These studies were unfortunately based on a comparison of BPH tissue that had been surgically resected with normal prostate tissue derived from cadavers. This debate was quelled to some extent by the publication of data comparing fresh BPH tissue with fresh normal prostate derived from live organ donors. These showed that there was no significant difference between the levels of DHT in normal and BPH tissues (Walsh et al., 1983). Work on canine BPH showed that this condition could be induced with androstanediol and with combinations of androstanediol and estradiol. A combined dose of DHT and estradiol was also found to induce the disease (DeKlerk et al., 1979b; Walsh and Wilson, 1976).

In man, levels of serum testosterone drop by about 35% between the ages of 21 and 85 against a constant level of estradiol. Thus there is a change in the androgen/estrogen ratio, which may be sufficient to promote the growth of BPH.

McNeal developed the important concept of reawakening of embryonic inductive potential in BPH stromal cells (McNeal, 1978, 1983, 1984, 1985). This idea is based on observations suggesting that growth of the prostate results from growth factors and chemokines acting between the prostatic epithelium and stroma (Jerde and Bushman, 2009; Marker et al., 2003). In the adult prostate a broadly homeostatic balance of growth promoting and growth inhibiting factors is presumed to maintain organ integrity (Hayward and Cunha, 2000). A localized breakdown of such a balance could result in focal re-growth of new prostatic tissues. Adult prostatic epithelium from rats, mice and humans can respond to prostatic inductive mesenchyme with new growth and development (Chung et al., 1984; Hayashi et al., 1993; Hayward et al., 1998; Norman et al., 1986), providing experimental support for this idea. However, this does not address the underlying cause of such a stromal to mesenchymal switch. The specific factors that mediate hyperplastic enlargement of the prostate remain to be determined, although it is likely that these will prove to be the same factors involved in normal prostatic growth given the disease does not normally progress to malignancy.

While there are some naturally occurring animal models of BPH, notably dogs and some non-human primates including chimpanzees (Steiner et al., 1999), the condition does not naturally occur in common laboratory models such as mice. Like its human counterpart, canine BPH increases in frequency with age and is restricted to animals with intact testicular function. Canine BPH is histologically and anatomically distinct from the human condition. The canine disease is usually diffuse, occurring throughout the gland (DeKlerk et al., 1979a; McNeal, 1984, 1985) in comparison to the human focal phenotype. A generalized expansion of the canine prostate that compresses the rectum and produces constipation is the common presentation. In contrast, focal growth of transition zone nodules in humans compresses the urethra and results in urinary retention.

5. Lower urinary tract symptoms (LUTS)

Enlargement of the prostate can alter lower urinary tract dynamics, resulting in a constellation of symptoms often referred to as lower urinary tract symptoms or LUTS. These may include storage related symptoms such as frequent urination, urgency of urination, waking up at night to void (nocturia) and urinary incontinence to voiding related symptoms such as hesitancy, straining to void, poor urinary stream and failure to properly empty the bladder. The link between BPH and LUTS is likely multifactorial and may include physical obstruction caused by the hyperplastic nodules impinging on the urethra, altered bladder neck compliance and tone, and/or altered detrusor smooth muscle physiology and function over time in response to altered or obstructed outflow. In addition, multiple other disease processes can also impinge on the storage and voiding function of the lower genitourinary tract that can either mimic or exacerbate the changes seen with BPH. These include processes such as neurologic disorders, malignancies and stricture disease. As a consequence, the relationship between the extent/severity of BPH and the degree of LUTS is not simple or linear and can be influenced by multiple factors in a complex, dynamic relationship.

Since BPH/LUTS is predominantly a disease of the elderly and given the progressive aging of the population in the United States, BPH/LUTS represents a significant and growing public health challenge. While estimates vary based on the definition used, the number of men requiring treatment for LUTS overall is approximately 16/1000 person years overall, increasing from 3.3/1000 for men aged 40–49 and rising up to 30/1000 person years for those over age 70 (Jacobsen et al., 1999). In 2005 alone, expenditures for treatment of BPH/LUTS related problems in the private sector were estimated at almost \$4 billion (Saigal and Joyce, 2005). Untreated, BPH can ultimately give rise to severe sequelae. Permanent bladder dysfunction/acontractility, urinary retention, severe renal dysfunction, end stage renal disease and death are all possible outcomes. However, the incidence of such consequences in the developed world is extremely low. In practice, men are evaluated and treated when BPH is an annoyance rather than a threat to life. Virtually all of the therapy for BPH/LUTS focuses on symptom management after the disease has manifested. Strategies that would prevent either the hyperplastic growth of the prostate or prevent the downstream manifestations and physiological changes leading to LUTS would be preferable, but are predicated on an in depth understanding of the mechanisms underlying these processes. To date, these mechanisms have not been fully defined.

6. Common co-morbidities affecting BPH/LUTS patients

The complex symptomatology of LUTS patients can involve a number of conditions, some of which may be causal to the disease

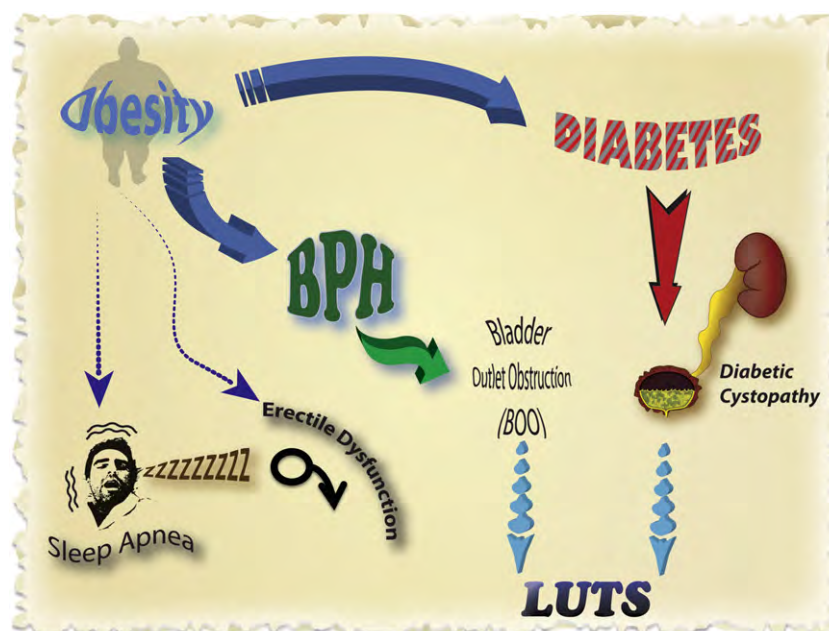


Fig. 2. Co-morbidities commonly seen in patients with benign prostatic hyperplasia and lower urinary tract symptoms (BPH/LUTS). Patients commonly, but by no means always, present with an array of symptoms that are interlinked. These include obesity, which can directly give rise to sleep apnea, erectile dysfunction and diabetes. BPH and LUTS can arise as a result of a number of these conditions. Sorting out cause and effect is a major clinical problem when trying to determine optimal therapies for individuals.

state while others may be a consequence of shared pathophysiological changes. As shown in Fig. 2 common co-morbidities of patients with LUTS include the metabolic syndrome, diabetes and obesity.

6.1. BPH/LUTS and the metabolic syndrome

Studies exploring the etiology of prostatic hyperplasia have traditionally focused on aberrations of the steroid signaling axis as a causal entity. More recently, however, there has been a growing appreciation that other systemic conditions that often co-exist in men as they age may play a causal role. A prime example of this is the metabolic syndrome. This syndrome is a profile of findings including impaired glucose metabolism, obesity, altered fat distribution, hypertension, dyslipidemia, markers of systemic inflammation such as elevated C-reactive protein and autonomic-sympathetic overactivity, which affects up to 50 million people in the United States (Kasturi et al., 2006; Rohrmann et al., 2005). A link between the metabolic syndrome and prostatic hyperplasia was suggested in a series of 158 men with LUTS where those men with larger prostates had increased incidence of diabetes, hypertension and obesity while also having lower levels of low-density lipoprotein (LDL) and higher serum insulin levels (Hammarsten et al., 1998). A follow up study showed similar findings in a cohort of 250 men with BPH/LUTS (Hammarsten and Hogstedt, 1999). The men with faster growing prostates had a higher prevalence of diabetes and treated hypertension as well as several measures of obesity and had higher serum insulin levels and lower levels of high-density lipoprotein (HDL). These co-morbidities are all elements of the metabolic syndrome. Several subsequent reports have substantiated the notion that at least some elements of the metabolic syndrome are more common in men with BPH/LUTS (Hammarsten and Hogstedt, 2001; Moul and McVary, 2010; Nandeesh et al., 2006a), though this was not universally true (Lekili et al., 2006; Zucchetto et al., 2005). In particular at least two of the elements linked to the metabolic syndrome, obesity and diabetes have been repeatedly associated with BPH/LUTS in men across multiple studies, though whether this relationship is causal remains to be proven.

6.2. Diabetes and BPH/LUTS

Possible links between prostatic hyperplasia and diabetes were noted as far back as in 1966 in early epidemiologic studies (Bourne and Griffin, 1966, 1968a, b). More recently the studies focusing on BPH/LUTS and diabetes can be broken down into three basic groups. The first are those that looked at the association with prostatic hyperplasia, typically expressed as radiographic measurements of prostate volume by transrectal ultrasound (TRUS) or magnetic resonance imaging (MRI) or using a surrogate for prostatic volume such as serum prostate specific antigen (PSA). The second are those that look at the clinical diagnosis of BPH, typically defined either as the need for medical or surgical therapy for BPH, documented obstruction or low urinary flow rate on urodynamics or other testing, or assignment of the diagnosis by a physician. The last looks not at hyperplasia but at the LUTS themselves most often through validated self-administered questionnaires such as the International Prostate Symptom Score (IPSS) or similar instruments. These groups represent some of the various possible definitions and end points that may be used for studying BPH/LUTS. Many studies have examined some or all of these potential end points and in many cases have mixed definitions or failed to clearly delineate which endpoint was the focus of the study. This represents just one of several reasons why there are some inconsistencies and controversies surrounding these associations. The other major problem is variations on what population was used as the control group, studies that were often underpowered and variations on how diabetes was defined for the purposes of the study. Nevertheless the weight of the evidence suggests there is a moderate association of diabetes with BPH and prostatic enlargement and a stronger association of diabetes with LUTS, supporting the concept that LUTS can be either dependent or independent of BPH.

Several studies have shown a significant relationship between diabetes or hyperinsulinemia and larger prostate size (Berger et al., 2006; Hammarsten and Hogstedt, 1999, 2001; Hammarsten et al., 1998; Nandeesh et al., 2006a; Parsons et al., 2006). For example, in one of the largest studies to address this question,

Parsons et al. (2006) studied 422 men in the Baltimore Longitudinal Study of Aging and found that increased fasting blood glucose levels and diabetes were both risk factors for larger prostates assessed by MRI. Hammarsten and Hogstedt (2001) followed 307 patients with clinical BPH using TRUS and found that larger annual prostatic growth was associated with several components of the metabolic syndrome including diabetes. They also found that total gland volume was positively associated with more advanced age and higher plasma insulin levels. Other studies, such as a study of 2115 men in Olmsted County, have not shown an association between diabetes and BPH (Burke et al., 2006). This work found that diabetes was associated with higher LUTS as measured by IPSS score and lower peak urinary flow rates, but not with prostate volume. It should be pointed out that the proportion of men in this cohort with diabetes was quite small, limiting the power of the study even though the overall population was large. Further, lower urinary flow rates and LUTS are used in many studies as criteria for diagnosing BPH for the purposes of epidemiological studies.

The association between diabetes and the clinical diagnosis of BPH has been made across a number of different studies (Berger et al., 2005; Burke et al., 2006; Dahle et al., 2002; Nandeesh et al., 2006a). The largest of these studies looked specifically at the clinical diagnosis of BPH (Dahle et al., 2002). Two hundred men who had undergone surgery for BPH were compared to 302 normal control men. The findings were that the men who had undergone surgery for BPH had higher plasma insulin levels and increased waist to hip ratio, an indication of truncal obesity. In another study, Berger et al. (2005) found that those with evidence for vascular damage due to diabetes were more likely to be diagnosed with BPH/LUTS. One study out of Scandinavia failed to find an association between BPH and diabetes, but this study is subject to significant recall bias since all diagnoses were self-reported (Koskimaki et al., 2001).

The strongest association between diabetes and BPH/LUTS has been across studies that have either focused specifically on LUTS or included this as part of the definition of BPH (Berger et al., 2005, 2006; Burke et al., 2006; Joseph et al., 2003; Michel et al., 2000;

Sarma et al., 2008; Zhang et al., 2008a). One of the largest is the cohort study from Olmsted County mentioned above, but which was focused predominantly on Caucasian men (Burke et al., 2006). The association between diabetes and LUTS has also been demonstrated in a cohort of over 700 African American men (Joseph et al., 2003), showing a link between diabetes and LUTS. Michel et al. (2000) analyzed over 9800 men with BPH, including 1290 with diabetes, and found that the diabetic men had worse IPSS scores and lower peak urinary flow rates than the non-diabetic men. The association has also been demonstrated when LUTS was more closely analyzed using various urodynamic parameters in a study of 166 men with BPH, 74 of whom also had diabetes (Zhang et al., 2008a). The diabetic men were more likely to demonstrate abnormalities with bladder capacity, decreased bladder sensation, poor bladder compliance and detrusor overactivity, while maximum detrusor pressure and evidence for bladder outlet obstruction were more common in non-diabetic men with BPH/LUTS.

6.3. Obesity and BPH/LUTS

As with diabetes there is a large body of evidence that supports a strong association between obesity and BPH/LUTS (Dahle et al., 2002; Fowke et al., 2007b; Giovannucci et al., 1994; Hammarsten and Hogstedt, 1999, 2001; Hammarsten et al., 1998; Kristal et al., 2007; Lee et al., 2006b, 2009a, b; Maserejian et al., 2009; Parsons, 2007; Parsons et al., 2006; Soygur et al., 1996; Xie et al., 2007); although this finding is not universal (Meigs et al., 2001; Seitter and Barrett-Connor, 1992; Zucchetto et al., 2005). This is particularly relevant given the known “epidemic” of obesity in the United States, which appears to be getting steadily worse. In addition, obesity is a potentially preventable or reversible health condition. If it could be demonstrated that it is causally linked to BPH/LUTS, then targeting obesity would represent a target for disease prevention (see Fig. 3). Such an intervention would also have multiple additional positive effects such as decreasing morbidity and mortality



Fig. 3. Potential mechanisms linking obesity and diabetes to benign prostatic hyperplasia and lower urinary tract symptoms (BPH/LUTS). A number of mechanisms relating to both obesity and diabetes can play a role in the development of BPH/LUTS, a few of which are outlined in this figure. Individual patients may be affected by more than one pathway.

secondary to insulin resistance and cardiovascular disease. As was the case with diabetes, reports have varied regarding the end points of BPH/LUTS that were studied. An additional layer of complexity in examining the studies of obesity, however, is that investigators have varied in their definition of obesity or examined more than one potential parameter. Examples of definitions of obesity have included simple body weight, body mass index (BMI), waist circumference, hip circumference and waist to hip ratio (WHR). Variations in both the end points of BPH/LUTS used in the study and the definition(s) of obesity account for some of the differences across studies. Nevertheless the preponderance of the evidence suggests a strong clinical association between measures of obesity and increased BPH/LUTS in general, and larger prostate volume in particular.

There has been a strong association found consistently across studies between measures of obesity and increased prostate volume (Fowke et al., 2007b; Hammarsten and Hogstedt, 1999, 2001; Hammarsten et al., 1998; Lee et al., 2006a, 2009a, b; Parsons et al., 2006; Soygur et al., 1996; Xie et al., 2007). Some of the larger studies have included the 422 men in the Baltimore Longitudinal Study of Aging, showing that obesity was associated with increased prostate size based on MRI (Parsons et al., 2006). A larger study by Fowke et al. (2007a) found that in 753 men with a negative TRUS prostate biopsy increased prostate volume by TRUS was associated with several measures of obesity, including increased BMI and waist circumference. Similar associations between obesity measures and larger prostate volume by TRUS have also been shown in a cohort of 649 Chinese men (Xie et al., 2007) and 602 Korean men (Lee et al., 2009a, b). In the latter study, the association was stronger in measures of central obesity (waist circumference) rather than overall obesity (such as BMI). The studies by Hammarsten and Hogstedt (1999, 2001), Hammarsten et al. (1998) demonstrating an association between more rapid prostate growth and elements of the metabolic syndrome have also shown that this includes an association with measures of obesity. The weight of the evidence strongly suggests, therefore, that obesity in general, and increased central obesity in particular, is associated with increased prostatic volume.

In one of the largest epidemiologic studies to address the issue of BPH and obesity the Health Professional Follow Up Study, which included over 25,000 men, found over a two-fold increased risk of requiring prostate surgery and a two-fold increased risk of having LUTS in men with increased waist circumference (a measure of central obesity) (Giovannucci et al., 1994). Another large-scale study based on the prospective Prostate Cancer Prevention Trial (PCPT) found that, among 5667 men who did not have BPH at baseline, those with a higher WHR were more likely to develop BPH/LUTS over the seven-year course of the trial (Kristal et al., 2007). Other studies have also corroborated these findings (Dahle et al., 2002; Lee et al., 2006b) including a study by Dahle et al. (2002) discussed previously, which found that men who had undergone surgery for BPH had a higher WHR compared to control men. It should be pointed out that two studies failed to find an association between obesity and BPH/LUTS (Meigs et al., 2001; Seitter and Barrett-Connor, 1992), but these were both smaller trials and therefore may have been underpowered to detect any association that may exist.

As with measures of prostate volume, the association between obesity and LUTS is generally a strong one. For example, the study by Giovannucci et al. (1994) of 25,892 men in the Health Professional Follow Up Study found a strong association between obesity and increased LUTS. Analysis of the PCPT trial also demonstrated increased LUTS in men with higher WHR (Kristal et al., 2007) and a study of Korean men found that waist circumference was positively associated with increased LUTS (Lee et al., 2009a, b). A small study of 68 men with BPH found

an association between obesity and prostate volume but not worse symptoms, although this was an underpowered study compared to those discussed previously (Soygur et al., 1996). Finally, approaching the question from a slightly different perspective, a report from Maserejian et al. (2009) on 1545 men from the Boston Area Community Health Survey found that increased total caloric intake was associated with higher LUTS. Overall then, the evidence suggests that direct or indirect measures of obesity are associated with increased prostate volume, LUTS and the clinical diagnosis of BPH/LUTS.

7. Mechanisms linking diabetes and obesity to BPH/LUTS

In considering the systemic effects of diabetes and obesity on BPH and LUTS it is important to differentiate between levels of effect. As discussed above, clinical studies have tended to group patients broadly. However, the causes of symptoms may well vary. For example LUTS can result from prostatic enlargement, causing urinary outflow obstruction by a static mechanism involving growth of epithelial and stromal elements. This gives rise to urethral compression (Berry et al., 1984; McNeal, 1984), versus an active mechanism in which $\alpha 1$ -adrenergic receptors are stimulated resulting in contraction of the urethra (Sarma et al., 2009). Apparently identical LUTS may also arise as a result of changes to bladder structure and innervation. In diabetic patients, for example, it can be difficult to differentiate between these different causes of symptoms. Diabetes can give rise to diabetic cystopathy, resulting in LUTS as a consequence of nerve damage, notably seen in both male and female patients (Hill et al., 2008; Kebapci et al., 2007; Rapidi et al., 2006). As a further complication there are also aging-related changes to the musculature and fibrotic nature of the detrusor, potentially also contributing to symptoms. Both diabetes and obesity can broadly be considered to be inflammatory states and inflammation is also a possible mechanism that can give rise to prostatic growth. The specific causes of similar patient outcomes are not well differentiated at present but are certainly important in selecting patients for therapy. Clearly, treating the prostate of a patient with diabetic cystopathy is unlikely to give rise to the best clinical outcome.

An examination of the various common co-morbidities suggests a hierarchy as shown in Fig. 3. Such an arrangement suggests that problems associated with obesity and its sequelae are prime drivers of BPH/LUTS. However, it must be acknowledged that while this represents the overall situation across the population, individual patient populations do not always fit neatly into such a model. Thus, while it may be that a “typical” patient is an overweight diabetic with an aversion to exercise, it is also true that patients can include slim, fit individuals. In terms of treatment of this diverse population, a clear understanding of the mechanisms underlying disease in a particular patient must be established and therapies then need to be selected on a personalized basis. Some of the major mechanisms involved in prostatic growth that are prospective therapeutic targets include changes in hormonal status, alterations in the insulin/IGF/IGF binding protein (IGFBP) axis, inflammation and alterations in cellular metabolism resulting from persistent abnormal stimulation.

7.1. Steroid hormones

The role of steroid hormones in the pathogenesis of BPH has been debated for several decades. Prostate development is absolutely dependent upon androgens, as discussed above, and disruption of the synthesis of dihydrotestosterone is a mainstay of BPH treatment. However, estrogens also affect prostatic development and histology. Classic studies by Price (1936) in the 1930s

demonstrated direct effects of estrogens on the prostate, including the induction of hyperplasia. Subsequent work has demonstrated that estrogens can act through estrogen receptor- α (ER α) located in the prostatic stroma to elicit epithelial squamous metaplasia at pharmacologic doses (Risbridger et al., 2001). Estrogens can also affect other hormones by changing the function of the hypothalamic–pituitary axis. The known and potential roles for estrogens in prostatic disease have been well reviewed elsewhere (Prins and Korach, 2008) and will be discussed briefly here in the context of BPH co-morbidities.

As noted above, changes in the testosterone/estradiol (T/E2) ratio seem to be a natural consequence of aging even in otherwise healthy men. Changes in sex steroid hormones associated with aging have been associated with alterations in body fat deposition producing an increased tendency towards central obesity. Such changes are outside the scope of this communication and have been reviewed elsewhere (Mayes and Watson, 2004). Here we are interested in the consequences of such fat deposition on the prostate. The enzyme aromatase, which acts to convert androgens to estrogens, is found in many tissues, notably in fat. In obese men large masses of body fat, with their associated aromatase activity, result in a significantly more estrogenic environment than seen in normal weight groups (Soygur et al., 1996). Neonatal estrogen exposure is a classic model for chronic prostatic inflammation in later life and has been shown to be effective even in otherwise estrogen-deficient mice (Bianco et al., 2006). In the context of BPH/LUTS, changes in the T/E2 ratio have been shown to be associated with inflammation and urodynamic changes in the adult Noble rat prostate (Bernoulli et al., 2007). The extent to which hormones versus inflammation cause such changes is not entirely clear (Bernoulli et al., 2008). These data certainly suggest the possibility that obesity might also play a causal role by altering hormonal status and thus triggering inflammatory changes in the prostate, which can contribute to BPH.

7.2. Insulin and IGF/IGFBP axis

Insulin resistance, a common precursor to type 2 diabetes, is closely associated with obesity and a high-fat diet (HFD; Kovacs and Stumvoll, 2005). This condition results in elevated fasting serum insulin levels and changes in glucose and lipid metabolism (reviewed in Vikram et al., 2010b). A number of drugs have been developed to treat insulin resistance, including Metformin and the PPAR γ -agonists—thiazolidinediones (TZDs). Insulin and the functionally related insulin-like growth factors have sequence homology and can cross talk through the same receptors. IGFs are associated with a series of IGF binding proteins which can either present or sequester ligand. The similarities in function between insulin and the insulin-like growth factor/IGFBP axis have led to a series of investigations into IGF activity in obese, diabetic and BPH patients. Alterations in IGFBP levels, specifically reductions in IGFBP-2, have been found in both BPH and obese patients (Cohen et al., 1994c; Nam et al., 1997). Increased levels of free serum IGF-1 have also been shown in obese patients, presumably due to changes in IGFBP levels (Nam et al., 1997). Epidemiologic links between BPH symptoms and elevated IGF-1 and decreased IGFBP3 have been reported in both Chinese and American patient cohorts (Chokkalingam et al., 2002; Neuhaus et al., 2008).

In vivo, IGF type I receptors are mainly expressed in prostatic epithelium, while the IGF-1 ligand is predominantly expressed in the stroma, supporting a paracrine role for these growth factors (Barni et al., 1994). Prostate stromal and epithelial cells in culture are sensitive to IGF and IGFBP levels (Cohen et al., 1994a, 1991), and these factors have also been linked to prostate cancer progression (Cohen et al., 1992, 1993; Tennant et al., 2003). Increased expressions of IGF-II and IGFBP-2 along with increased

IGFBP-5 expression have been described in BPH-derived stromal cells, suggestive of a potential link to the disease process (Cohen et al., 1994b). Prostatic development fails to occur properly in mice lacking either IGF-1 or the IGF type 1 receptor (Hayward and Cunha, 2000). In tissue rescue experiments using urogenital sinus from these animals, tiny but well differentiated prostates form. Increased levels of insulin have been shown to increase prostate cell proliferation in rats (Vikram et al., 2010a). The 5 α -reductase inhibitor Finasteride, which inhibits the conversion of testosterone to the biologically active metabolite DHT, is used to treat BPH, and has been shown to increase levels of IGFBP-5 in both humans and rats, concurrent with prostatic involution (Thomas et al., 1998, 2000). Thus a number of lines of evidence support the idea that changes in the IGF/IGFBP axis, consequent to obesity or diabetes, could play a role in regulating prostatic growth.

7.3. Inflammation

Increased inflammation is closely associated with the severity of BPH. Data from the MTOPS study suggest the risk of BPH progression and acute urinary retention is greater in men with prostatic inflammation (McConnell et al., 2003; McVary, 2007). Inflammation has also been linked to the development of hyperplasia. In a mouse model of chronic prostatitis, multiple regions of epithelial hyperplasia and dysplasia were found next to areas of inflammation (Elkahwaji et al., 2007). We generated a conditional knockout of PPAR γ by crossing probasin-cre and PPAR γ^{lox} mice to generate mice in which PPAR γ was deleted in the luminal epithelial cells. As shown in Fig. 4, prostatic hyperplasia, a common observation in these animals, is associated with inflammation in the mouse prostates (Jiang et al., 2010b).

In BPH patients, stromal nodules have been found to contain increased numbers of T and B lymphocytes (Bierhoff et al., 1996). Elevated levels of inflammatory cells were also detected in the interstitium and surrounding epithelial glands of human BPH tissues (Theyer et al., 1992). In this study the majority of inflammatory cells (60%) were CD4+ helper T cells with the remainder consisting of CD8+ cytotoxic T cells (30%) and B cells (10%). Additional studies revealed that T cells present in human BPH samples were chronically activated (Steiner et al., 1994). In BPH, infiltration of inflammatory cells is accompanied by increased expression of pro-inflammatory cytokines. Elevated levels of the interleukins IL-2, IL-8, IL-17 and interferon gamma (IFN γ) have been detected in BPH samples (Giri and Ittmann,

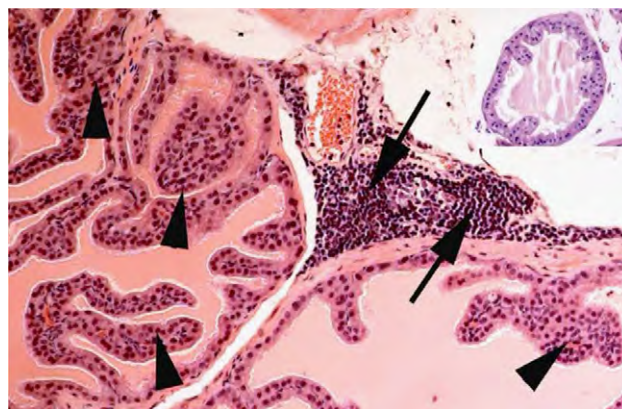


Fig. 4. Hyperplasia and inflammation in the anterior prostate of the conditional PPAR γ knockout mouse at 4 months of age. Inflammatory infiltrate (arrows) and various degrees of hyperplastic growth (arrowheads) in the prostate of a 4 month old PPAR γ^{PREKO} mouse. Note inflammatory infiltration outside of blood vessels and adjacent stromal changes. **Inset:** Age matched wild type control duct; note lack of inflammation and absence of hyperplastic changes.

2001; Kramer et al., 2002; Steiner et al., 2003). In addition, expression of IL-15, a cytokine that promotes T cell proliferation, and its receptor IL-15R were higher in BPH epithelial cells compared to normal prostate (Handisurya et al., 2001). Prostatic inflammatory atrophy also results in increased expression of a number of cytokines and inflammatory enzymes, including cyclooxygenase-2 (COX-2; De Marzo et al., 2007; Palapattu et al., 2005). Interleukins have recently been implicated as direct regulators of organ growth in the mouse prostate. IL-1 was shown to elicit IGF-dependent prostatic growth and reactive hyperplasia in a murine model (Jerde and Bushman, 2009). Increased cytokine expression influences the proliferation of non-inflammatory cells in the prostate, as demonstrated by the cytokines IL-2 and IFN γ , which have been shown to increase proliferation of human BPH stromal cell lines (Kramer et al., 2002). Increased inflammation can result in disruption of epithelial glands, with increased serum levels of PSA. Agents that reduce inflammation could also be effective at reducing hyperplasia, thus reducing the symptoms associated with this disease.

A small number of studies have been conducted and have demonstrated some efficacy of anti-inflammatory strategies in patients with BPH (Azzouzi et al., 2006). For example, loxoprofen at 60-mg dose in patients with LUTS resistant to a first-line treatment showed an improvement of the nocturia in 74% of the cases with better results in more severe cases (Araki et al., 2004). Of particular interest, the combination of a COX-2 inhibitor and a 5 α -reductase inhibitor resulted in increased apoptosis in BPH (Sciarra et al., 2008). Di Silverio et al. (2005) tested Rofecoxib (a COX-2 inhibitor) with Finasteride (a 5 α -reductase inhibitor) versus Finasteride alone. Their hypothesis was that an anti-inflammatory drug could palliate the lack of efficiency of the Finasteride during the first week of treatment. The authors did find short-term relief in symptomatic scores. Links between specific mediators of inflammation and cellular metabolism may represent a critical direction in which new approaches for BPH therapies can be directed. Based upon the data presented above, we would hypothesize that an appropriate anti-inflammatory approach could additionally provide a long-term complementary effect to the 5 α -reductase inhibitor. PPAR γ is a possible candidate for managing prostate growth, given its demonstrated efficacy at regulating insulin sensitivity in other insulin target tissues. To date, there are no retrospective analyses of BPH incidence for diabetic patients taking TZDs.

8. Peroxisome proliferator-activated receptor gamma (PPAR γ) signaling

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily (Auwerx et al., 1996; Evans et al., 2004). The PPAR family is composed of PPAR α (NR1C1), PPAR β/δ (NR1C2) and PPAR γ (NR1C3) (Barish and Evans, 2004; Evans et al., 2004; Lee et al., 2003; Roberts et al., 2003). PPARs bind as obligate heterodimers with the retinoid X receptors (RXRs) to cognate DNA elements named peroxisome proliferator response elements (PPREs; Evans et al., 2004). PPARs regulate lipid and glucose metabolism (Argmann et al., 2005; Evans et al., 2004; Forman et al., 1996; Metzger and Chambon, 2007). They also participate in cell growth, proliferation and differentiation in adipocytes, hepatocytes and keratinocytes as well as prostate epithelial cells (Chambon, 2005; Imai et al., 2001a; Imai et al., 2001b; Jiang et al., 2004; Metzger et al., 2005). The PPAR/RXR/PPRE interaction leads to the recruitment of coactivators and the general transcriptional machinery, resulting in alterations in gene transcription and cell function (Rosen and Spiegelman, 2001). There are two

forms of PPAR γ , denoted PPAR γ 1 and PPAR γ 2. These represent proteins derived from alternative transcriptional start sites in the same gene, giving rise to two isoforms. PPAR γ 2, using the earlier start site, has an additional 30 amino acid residues (Fajas et al., 1997; Tontonoz et al., 1994b).

PPAR γ protein levels are the highest in adipose tissue, where PPAR γ ligands induce adipocyte differentiation and regulate lipid storage and metabolism (Theocharis et al., 2004). In addition, PPAR γ functions as an important regulator of cell differentiation, proliferation and apoptosis in other types of stromal cells (macrophages, endothelium and smooth muscle; Barak et al., 1999; Rosen and Spiegelman, 2001; Tontonoz et al., 1994a) and parenchymal epithelial cells of the breast (Yee et al., 2003), colon (Saez et al., 1998; Sarraf et al., 1998) and prostate (Shappell et al., 2001a, 2001b). Ligands for PPAR γ include the eicosanoids 15S-Hydroxyeicosatetraenoic acid (15-HETE; Bhatia et al., 2003; Shappell et al., 2001b) and the prostaglandin 15-deoxy- Δ 12,14-prostaglandin J2 (15dPGJ2; Matsuyama et al., 2005; Soares et al., 2005), docosahexaenoic acid (DHA; Yu et al., 2008), polyunsaturated fatty acid (PUFA; Calder, 2008) and nonsteroidal anti-inflammatory drugs (NSAID; Romeiro et al., 2008) as well as synthetic thiazolidinedione (TZD) drugs (Rosen and Spiegelman, 2001). The TZDs rosiglitazone and pioglitazone are currently used in the clinic to treat patients with type II diabetes, due to their ability to increase insulin sensitivity by stimulating glucose uptake (Smith and Kantoff, 2002). However, use of these drugs has been severely curtailed of late because of cardiovascular side effects and also because clinical trials are showing little improvement over more established therapies for diabetes treatments (Lindberg and Astrup, 2007). To address the side effects in off-target tissues, a more thorough understanding of the function, dietary regulation and tissue-specific stoichiometric distribution of each isoform is necessary.

PPAR γ plays a key role in regulation of cellular lipid metabolism, redox status and organelle differentiation in adipose tissue and other organs, including prostate (Jiang et al., 2004; Sugii et al., 2009). The prostaglandin 15dPGJ2 has been described as a PPAR γ activator and may potentially play an anti-inflammatory role in a PPAR γ -dependent manner, decreasing COX-2, PGE2 synthase (PGES) and PGE2 production (Mendez and LaPointe, 2003). Prostate cancer is characterized by increases in pathways that generate pro-inflammatory prostaglandins and by a loss of enzymes in lipid metabolic pathways such as arachidonate 15-lipoxygenase-2 (15-LOX-2), which generate natural ligands for PPAR γ (Shappell et al., 2001b). In contrast, the common co-morbidities associated with BPH/LUTS elicit changes in the prostatic metabolic environment, specifically with increased lipid availability and alterations in glucose metabolism consequent to insulin resistance and changes in the IGF/IGFBP axis. Loss of PPAR γ function in the prostate leads to a number of consequences, including widespread inflammation and hyperplastic growth (Jiang et al., 2010b), with more focal premalignant changes. Due to its central position in balancing cellular metabolism and differentiation, and to the existence and current clinical use of PPAR γ agonists, PPAR γ is an attractive target for manipulation in therapeutic strategies to treat prostatic disease.

8.1. Transcriptional regulation of PPAR γ signaling

PPAR γ consists of distinct functional domains including an N-terminal transactivation function domain (AF-1), a highly conserved DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) that contains a ligand-dependent transactivation function (AF2). Upon ligand binding the complex of PPARs and RXRs binds to specific recognition sites on DNA, the peroxisome proliferator response elements (PPREs), and regulates gene transcription. A PPRE, consisting of an almost perfect direct

repeat of the sequence TGACCT spaced by a single base pair, has been identified in the upstream regulatory sequences of genes related to metabolic and cell cycle-regulating pathways. Activation of PPAR γ results in increased expression of many target genes involved in lipid metabolism and energy balance such as the adipocyte fatty acid binding protein (aP2), acyl-CoA oxidase, lipoprotein lipase, acyl-CoA synthase and adiponectin (Memon et al., 2000).

In adipose tissue of obese mice the expression of catalase, an anti-oxidant enzyme, is significantly decreased, potentially resulting in insufficient elimination of hydrogen peroxide. Adipose catalase expression is regulated via a novel remote PPAR γ -responsive region. Treatment of mice with PPAR γ agonists significantly enhanced catalase expression in adipose tissue (Okuno et al., 2008). We have demonstrated a similar loss of catalase expression resulting from PPAR γ knockout in mouse prostate epithelium (Jiang et al., 2010b).

8.2. Phosphorylation of PPAR γ protein

Mitogen-activated protein kinase (MAPK) signaling remains the focus of intensive research because it plays a pivotal role in mediation of cellular responses to a variety of signaling molecules (Kohno and Pouyssegur, 2003). Among the key signaling pathways that regulate mammalian cell growth and differentiation are the MAPK kinases (MEK)/extracellular signal-regulated kinase (ERK), comprised of MAP kinase kinases, MKK1/2 or MEK1/2, and MAP kinases ERK1/2. MEK1/2 and ERK1/2 are acutely stimulated by growth and differentiation factors in pathways mediated by receptor tyrosine kinases, heterotrimeric G protein-coupled receptors or cytokine receptors, primarily through p21Ras-coupled mechanisms. These enzymes are ubiquitous and generally expressed at micromolar levels in mammalian cells (Burgermeister and Seger, 2008; Kohno and Pouyssegur, 2003).

A number of the molecular mechanisms activated in BPH regulate intracellular MAP kinase signaling pathways in epithelial and/or stromal cells. P38 MAP kinase is not normally activated in the prostatic epithelium. However, activation is seen in both BPH and prostate cancer (Royuela et al., 2002). Of particular relevance here, the IGF pathway acts to activate MAP kinase signaling in prostatic epithelial cells as do a number of inflammatory chemokines produced by the lymphocytic infiltrate commonly found in BPH (Theyer et al., 1992). MAPK regulation in BPH is also important in stromal cells. Estrogenic signaling through the stromal ER α , which is implicated in the development of BPH, stimulates proliferation of the stromal cells via activation of ERK (Zhang et al., 2008b), and flavonoids such as apigenin can inhibit stromal cell proliferation via decreases in ERK1 and 2 (Bektic et al., 2006). Phosphodiesterase inhibitors have also recently been shown to elicit clinical responses in BPH patients (initially seen as a side effect of their use in erectile dysfunction—reviewed in Laydner et al., 2011). This effect has also been shown to result from regulation of stromal MAPK signaling (Zenzmaier et al., 2010). The role of MAPK signaling in the prostate has been thoroughly reviewed elsewhere (Maroni et al., 2004; Papatsoris and Papavassiliou, 2001).

PPAR γ is a downstream target of MEK/ERK signaling (Burgermeister and Seger, 2007; Burns and Vanden Heuvel, 2007; Diradourian et al., 2005). A unique MAPK phosphorylation site was mapped at serine 82 in the N-terminal domain in mouse PPAR γ 1, which corresponds to serine 112 of mouse PPAR γ 2. Substitution of this serine by alanine led to a loss of PDGF-mediated repression of PPAR γ activity (Shao et al., 1998). Serine 84 of PPAR γ 1 is phosphorylated by ERK2- and JNK-MAPK in humans (Camp et al., 1999). This phosphorylation is totally abolished either by mutation of serine 84 to alanine or co-expression of a phosphoprotein phosphatase. Similar to mouse

PPAR γ , human PPAR γ 1 phosphorylation inhibits both ligand-dependent and -independent transactivating functions whereas an S84A mutant shows an increase in AF-1 transcriptional activity of PPAR γ (Adams et al., 1997). We performed gene expression profiling on both PPAR γ - and γ 2-deficient mouse prostatic epithelial (mPrE) cells and found the up-regulation of numerous genes in the MAPK signaling pathway (Jiang et al., 2010b), suggesting that MEK/ERK signaling may function as a key mediator of the phosphorylation status of PPAR γ protein. It has been also reported that PPAR γ ligands can activate MAPK signaling via non-genomic signaling (Gardner et al., 2005; Hedvat et al., 2004). However, the precise molecular cross-talk between PPAR γ and MEK/ERK signaling is still unclear.

Choi et al. (2010) showed that the cyclin-dependent kinase 5 (Cdk5) is activated in high-fat-fed obese mice, resulting in phosphorylation of PPAR γ at serine 273. This modification of PPAR γ does not alter its adipogenic capacity but leads to dysregulation of a large number of genes whose expression levels are altered in obesity, including reduced adiponectin. Interestingly the phosphorylation of PPAR γ by Cdk5 is blocked by anti-diabetic PPAR γ ligands such as rosiglitazone and MRL24, and is completely independent of classical receptor transcriptional agonism. The authors suggest Cdk5-mediated phosphorylation of PPAR γ may be involved in the pathogenesis of insulin resistance and present an opportunity for development of an improved generation of anti-diabetic drugs working through PPAR γ .

8.3. PPAR γ signaling and oxidative stress

Oxidative stress increases with age and may be aggravated with any pathological condition that damages tissues, resulting in additional complications. Chronic inflammation and associated oxidative stress are commonly associated with BPH and LUTS. Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, the so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism (Durackova, 2010). Antioxidants are substances that are able to compete with the other oxidizable substrates and thus significantly delay or inhibit their oxidation. Enzymatic antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), and non-enzymatic antioxidants, including glutathione and vitamin C, play a role as scavengers of free radicals. Continued oxidative stress can lead to chronic inflammation, which in turn could mediate many chronic diseases, including cancer, diabetes, cardiovascular, neurological and pulmonary diseases, as well as benign prostatic growth. Oxidative stress can activate a variety of transcription factors, including p53, PPAR γ , NF κ B, AP-1, HIF-1 α , β -catenin/Wnt and Nrf2, leading to the altered expression of many genes, including those for growth factors, inflammatory cytokines, cell cycle regulatory molecules, and anti-inflammatory molecules many of which are dysregulated in BPH.

8.4. PPAR γ signaling in inflammation and immunity

PPAR γ sits at a crucial balance point in regulating oxidative stress within the cell. PPAR γ expression has been detected in human and/or mouse macrophages, dendritic cells, T and B cell lymphocytes, natural killer cells, mast cells, neutrophils and eosinophils (Szeles et al., 2007). Activation of PPAR γ in these cells not only results in expression of gene products involved in lipid metabolism, but also produces anti-inflammatory effects. The anti-inflammatory responses induced by PPAR γ ligands appear to be the product of altered immune cell protein expression

and function. PPAR γ ligands reduce recruitment of neutrophils, monocytes and eosinophils (Standiford et al., 2005; Woerly et al., 2003). They have also been reported to inhibit expression of the chemokine receptor CCR7, which regulates recruitment of dendritic cells (Szeles et al., 2007). PPAR γ ligands reduce induction of inflammatory cytokines TNF α , IL-1b and IL-6 by the phorbol ester PMA (Jiang et al., 1998) and inhibit expression of inducible nitric oxide synthase (iNOS) and COX-2 (Chawla et al., 2001). Thus, in BPH, PPAR γ may function in both epithelial and inflammatory cells to influence cell recruitment, cytokine production and survival.

PPAR γ can act to inhibit NF- κ B activation. Following translocation of the RelA-p50 complex to the nucleus, RelA-p50 competes with activated PPAR γ for a rare co-activator PPAR γ co-activator-1 (PGC1). Activation of PPAR γ by ligand allows binding of PPAR γ to PGC1. This means that PPAR γ competes with the RelA-p50 complex for a co-activator, allowing activation of PPAR γ to suppress NF- κ B activity (Wang et al., 2007). Loss of PPAR γ function as a result of an inflammatory environment can thus enable production of inflammatory cytokines. In addition, PPAR γ -induced inhibition of inflammation may be linked to reduced NF- κ B signaling. Administration of TZDs or PPAR γ expressing adenovirus reduced nuclear levels of the NF- κ B subunit p65/RelA within mouse lungs exposed to the irritant ovalbumin (Lee et al., 2006a).

Consistent with its reported effects on inflammatory cells, PPAR γ activation has been shown to reduce inflammation in multiple rodent models. In a mouse model of asthma induced by ovalbumin administration, treatment with the TZDs rosiglitazone, pioglitazone or an adenovirus expressing PPAR γ resulted in a decrease in the number and infiltration of inflammatory cells (Lee et al., 2005). Rosiglitazone has also been shown to reduce acute pancreatitis induced by cerulein (Ivashchenko et al., 2007) and inflammation in the dextran sodium sulfate model of colitis (Katayama et al., 2003). Furthermore, TZDs troglitazone and ciglitazone inhibit phorbol ester-induced cutaneous inflammation in mice (Mao-Qiang et al., 2004). The anti-inflammatory effects of PPAR γ agonists are likely due in part to altered expression of pro-inflammatory cytokines. TZDs have been reported to reduce expression of the interleukins IL-3, IL-4, IL-5 and IL-8 as well as TNF α in inflamed tissues (Katayama et al., 2003; Lee et al., 2005; Nakajima et al., 2001). Furthermore, co-administration of PPAR γ adenovirus with the TZD rosiglitazone has been reported to induce expression of the anti-inflammatory cytokine IL-10 in colon (Katayama et al., 2003).

There is a strong relationship between PPAR γ action and COX-2 expression. Analysis of 45 human BPH samples revealed COX-2 was elevated in glands with increased inflammation, specifically T cell and macrophage infiltration (Wang et al., 2004). PPAR γ suppresses COX-2 expression in human prostatic epithelial cells (Sabichi et al., 2004). Direct transcriptional regulation of COX-2 by PPAR γ has been reported (Subbaramaiah et al., 2001). This is consistent with our own (unpublished) *in vitro* observations that in prostatic epithelial cells in which PPAR γ expression is knocked out, transcripts of COX-1 increase 3 fold and of COX-2 approximately 6 fold. As discussed previously, COX-inhibitors have been tested clinically in BPH patients in combination with Finasteride with promising results in a small and short study (Di Silverio et al., 2005).

PPAR γ is expressed at high levels in circulating human monocytes and its activation increases the expression of macrophage-specific markers, such as CD14 and CD11b (Tontonoz et al., 1998). Inactivation of PPAR γ in macrophages results in the development of significant glucose intolerance plus skeletal muscle and hepatic insulin resistance in lean mice fed a normal diet. The relative degree of insulin resistance became more severe in mice lacking macrophage PPAR γ following high-fat feeding. This suggested that macrophage PPAR γ is required for normal skeletal muscle and hepatic insulin sensitivity and full anti-diabetic effects of

TZDs (Hevener et al., 2007). Pascual et al. (2005) reported the identification of a molecular pathway by which PPAR γ represses the transcriptional activation of inflammatory response genes in mouse macrophages. The initial step of this pathway involves ligand-dependent SUMOylation of the PPAR γ ligand-binding domain, which targets PPAR γ to nuclear receptor corepressor (NCoR)-histone deacetylase-3 (HDAC3) complexes on inflammatory gene promoters. This in turn prevents recruitment of the ubiquitination/19S proteasome machinery, which normally mediates the signal-dependent removal of corepressor complexes required for gene activation.

8.5. PPAR γ , lipotoxicity and diabetes

There has been a 4- to 8-fold increase in the prevalence of diabetes mellitus in the United States since the 1950s (Engelgau et al., 2004) with some models estimating that by 2050 there will be over 29 million individuals diagnosed with diabetes mellitus in the United States (Boyle et al., 2001). TZDs such as rosiglitazone and pioglitazone were widely prescribed in clinical practice to improve health outcomes in patients with diabetes mellitus (DM). This pharmacological class functions as potent PPAR γ ligands. TZDs were approved for marketing in the United States in 1999 and in FY 2007 over 7.4 million prescriptions were written for TZDs, with pioglitazone being the most commonly prescribed (Alexander et al., 2008). Clinical trials have demonstrated that at maximal doses these agents can reduce glycosylated hemoglobin A1C (HbA1c) by 0.7–1.6%, while the addition of a TZD to metformin, sulfonylurea or insulin therapy can reduce HbA1c by 0.8–1.5% (Aronoff et al., 2000; Fonseca et al., 2000; Kipnes et al., 2001; Lebovitz et al., 2001; Scherbaum and Goke, 2002). A recent meta-analysis suggested that rosiglitazone may be associated with increased cardiovascular risk (Nissen and Wolski, 2007); however, similar studies have not reached the same conclusion (Home et al., 2007; Lago et al., 2007). Although these safety concerns regarding rosiglitazone have resulted in a 50% decrease in rosiglitazone prescriptions, close to 30% of these former rosiglitazone users are being shifted to pioglitazone. These concerns underline the need for different classes of drugs to influence the PPAR γ signaling axis. The potential relevance of these drugs to prostate growth is underlined by animal studies showing that insulin-resistant rats given pioglitazone showed improved insulin sensitivity, decreased plasma insulin levels and decreased prostate weight (Vikram et al., 2010a).

When the limits of adipocyte expansion are reached at morbid obesity, ectopic lipids accumulate in secondary organ sites causing dyslipidemia, lipotoxicity and inflammation usually concurrent with insulin resistance. Typically, sequelae associated with obesity include type II diabetes, nonalcoholic fatty liver and cardiovascular disease (Virtue and Vidal-Puig, 2010). Type 2 diabetes is the result of systemic insulin resistance and can result in hyperinsulinemia in a chronic setting. Even in non-diabetic patients, hyperinsulinemia and dyslipidemia are independent risk factors for BPH (Nandeesha et al., 2006b). Recent studies suggest that TZD-induced insulin sensitivity in skeletal muscle is accomplished through alleviation of local hyperlipidemia by storage of excess fatty acids in intramuscular adipocytes. The pathophysiological links between insulin sensitivity and lipotoxicity are now well recognized, and are particularly relevant to the therapeutic ability of TZDs (DeFronzo, 2010).

Although hundreds of TZD target genes have been identified by microarray analysis of various tissues from TZD-treated animals, the specific genes responsible for the induction of insulin sensitivity remain unclear (Hsiao et al., 2011). Little work has been done to date on the function of the individual isoforms of PPAR γ in prostate but the consensus in other tissues (e.g. adipose and muscle) seems to be that PPAR γ 2 is responsible for the

reduction of lipotoxicity through the metabolism of excess lipotoxic fatty acids into triglycerides and phospholipids (Medina-Gomez et al., 2007) whereas the function of PPAR γ 1 seems to be related to mitochondrial biogenesis and branched chain amino acid metabolism, which are also pathogenically linked to lipotoxicity (Hsiao et al., 2011; Sears et al., 2009).

In summary, systemic metabolic disease is associated with local inflammation, insulin resistance, dyslipidemia and lipotoxicity. The array of tissues affected by these sequelae to obesity continues to grow and may include prostate. Because the prostate is a known insulin target tissue and TZDs increase insulin sensitivity, a retrospective analysis of diabetic patients taking TZDs or metformin will reveal whether BPH/LUTS should be categorized and treated as a secondary site of insulin resistance/lipotoxicity like liver and muscle or insulin/inflammation-dependent growth as a side effect of hyperinsulinemia.

9. Application of cell lines and animal models in the studies of pathogenesis of BPH

Investigation on the bio-pathogenesis of benign prostatic hyperplasia (BPH) has been hampered by the fact that there are few appropriate cell lines or relevant animal models. The prostates of male mammals show quite marked species-specific differences in morphology and functions, and at present none of the models developed are completely satisfying.

9.1. Cell lines and tissue recombination–xenografting

Prostate research has suffered for many years from a lack of cell lines representing normal and pathologically identifiable disease states. In the case of benign prostate, disease cells should be able to recapitulate normal aspects of prostatic development. Recently, two new human adult non-tumorigenic prostatic epithelial cell lines NHPRE1 and BHPRE1 were spontaneously immortalized and characterized in our laboratory (Jiang et al., 2010a). NHPRE1 cells were designed as putative progenitor cells, showing high expression levels of stem cells-associated proteins CD133, CD44, OCT4 and PTEN detected by immunofluorescence (IF) staining and Western blotting. However BHPRE1 were defined as intermediate or transit amplifying (TA) cells, which highly expressed cell cycle regulation-related biomarkers p63, p53, p21/WAF1 and Rb. We have utilized a tissue recombination–xenografting mouse model to functionally compare regeneration of human prostatic glandular tissues *in vivo*. A series of tissue recombinants, made by mixing different ratios of human prostatic epithelial progenitor or intermediate cells and inductive rat urogenital sinus mesenchyme (UGM), were grafted to the renal capsule of immunodeficient SCID male mice. Both NHPRE1 and BHPRE1 cell lines were able to regenerate benign secretory ductal–acinar architecture *in vivo*, containing intact basal and luminal epithelial layers. Prostate differentiation-associated proteins, such as AR, PSA, NKX3.1 and 15-LOX-2, were appropriately expressed in the remodeling epithelia. As few as 10 NHPRE1 cells were able to regenerate benign prostatic glandular structure while 200,000 BHPRE1 cells were required to achieve prostatic architecture post-grafting at the same time course. These results suggest a greater proportion of progenitor/stem cells in NHPRE1 cells than in BHPRE1 cells consistent with the idea that *in vivo* remodeling of benign human prostatic ductal–acinar architecture is more efficient when initiated from adult tissue-specific putative progenitor cells rather than intermediate or TA cells.

The NHPRE1 and BHPRE1 cell lines are important for a number of reasons. First, their different molecular characteristics point to the potentially differing capabilities of cells holding different

stem/progenitor cell-associated abilities to appropriately express key biomarkers (including AR, PSA, NKX3.1 and 15-LOX-2) in a functional tissue recombination–xenografting model. These two cell lines regenerate appropriate benign human prostatic ductal–acinar architecture with both luminal and basal epithelial subpopulations that express the appropriate cytokeratins and prostate-related differentiation biomarkers. Such characteristics are either totally absent, or not appropriately presented in the human prostatic cell lines that were previously available to the research community. These cells are fully benign as determined by histopathologic grading. This also makes them potentially useful models to compare molecular and cellular biological mechanisms in benign and malignant human prostate diseases.

9.2. Animal models

There are a limited number of murine models of BPH. A prostate-specific 15-LOX-2 transgenic mouse generated using the ARR2PB promoter enabled targeted expression of 15-LOX-2 or 15-LOX-2sv-b, a splice variant that lacks arachidonic acid-metabolizing activity, and resulted in age-dependent prostatic hyperplasia and enlargement of the prostate (Suraneni et al., 2010). This mouse model is consistent with the idea that the 15-LOX-2 product, 15-HETE, might be a biological ligand activating PPAR γ in humans (Shappell et al., 1999, 2001b).

Non-obese diabetic (NOD) mice are used as an animal model for type I diabetes. The mice exhibit susceptibility to spontaneous development of autoimmune insulin dependent diabetes mellitus (IDDM) and were first reported in 1980 (Makino et al., 1980). Histochemical and ultrastructural alterations in the ventral prostate (VP) of diabetic mice have been reported. The results showed reduction of the epithelia and increased stroma, with muscular and collagen hypertrophy in the prostatic gland and inflammation (Ribeiro et al., 2006). We have characterized a strong benign hyperplasia phenotype with lipid-like vesicle accumulation in the luminal epithelial cells of the anterior prostate (AP) of NOD/SCID mice at the age of 6.5 months. The ventral prostate (VP) of NOD/SCID mice also showed epithelial hyperplasia (Fig. 5).

The possibility of a dietary basis for BPH has been suggested by the disparate prevalence between the Western hemisphere and the Far East (Ranjan et al., 2006). The effects of dietary fatty acid quality on the rat ventral prostate growth, tissue organization and expression of AR and PPAR γ suggested that PPAR γ might represent a link between diet, prostate growth and AR expression and function (Escobar et al., 2009). The high-fat diet (HFD)-fed mouse is a model for studying mechanisms and treatment of impaired glucose tolerance and type II diabetes. The HFD-treated Sprague-Dawley rats showed prostatic enlargement by nine weeks (Vikram et al., 2010a). Significant increases in the cell proliferation markers also confirmed the occurrence of cellular hyperplasia in the prostate of hyperinsulinemic rats. Pioglitazone treatment led to improved insulin sensitivity, decreased plasma insulin level and prostate weight, indicting the role of compensatory hyperinsulinemia in prostate growth (Vikram et al., 2010a, 2010b).

10. Summary

In this review we have presented BPH in the context of common co-morbidities. We would suggest that these systemic conditions can contribute significantly to BPH/LUTS and that in some cases treatments aimed at co-morbidities will have beneficial side effects on prostatic pathophysiology. Many of the aforementioned metabolic diseases are now accepted co-morbidities for BPH, which may implicate the prostate as yet another innocent bystander of obesity, hyperinsulinemia and inflammation.

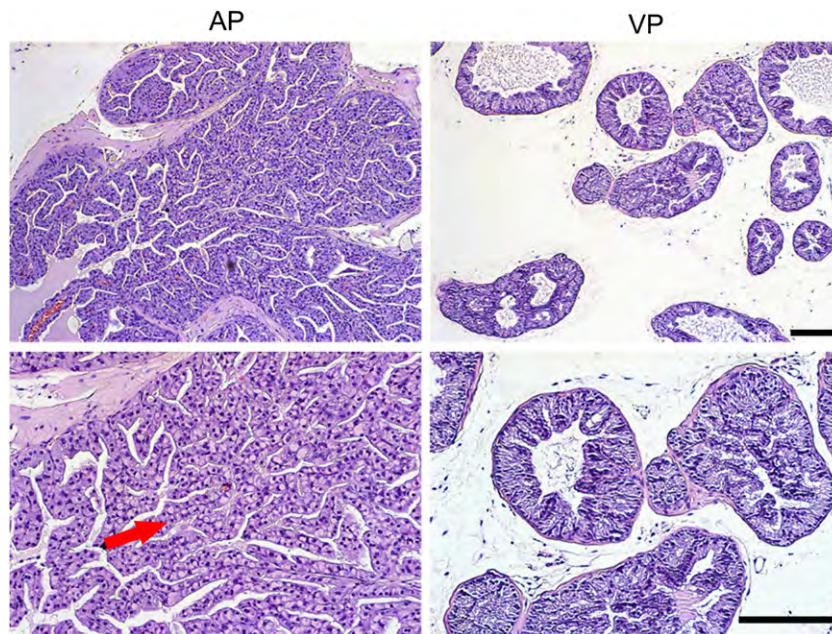


Fig. 5. Hyperplasia in the diabetic mouse prostate. H&E staining of the anterior (AP) and ventral (VP) prostatic lobes from 6.5 month old NOD/SCID male mice. A dramatic hyperplasia is seen in the anterior lobe with lipid-like vesicle accumulation in the luminal epithelial cells (arrow) and a milder hyperplasia occurs in the ventral lobe. Scale bars = 100 μ m (upper) and 50 μ m (lower) in the panels.

Current treatments directed at BPH do not address systemic metabolic disease. In addition, only recently have studies of co-morbidities for BPH addressed prostate volume. For example statins have been widely used to prevent cardiovascular disease, but recent studies show that these drugs also reduce BPH/LUTS as a beneficial side effect (St Sauver et al., 2011). We would suggest that the pathogenesis of BPH is a molecular subset of co-morbidities within the individual patient and may require unique or combinatorial treatments targeting the causative metabolic dysfunction specific to that patient.

We propose here that TZD-mediated regulation of insulin sensitivity, lipotoxicity and inflammation may improve prostate health either directly in the prostate or indirectly by modulating systemic co-morbidities. However, given the potential side effects of certain TZDs, more successful targets of the same glucose and lipid metabolic pathways should be explored.

Given the range of underlying systemic and molecular changes that can result in BPH/LUTS, the best treatment for patients with similar symptom profiles may vary. New approaches that treat common co-morbidities may well have clinical outcomes that impact these secondary symptoms in addition to their primary intended targets. If appropriately examined the results of such studies could potentially make valuable contributions toward the development of individualized treatment plans for BPH/LUTS patients, and toward understanding the pathophysiology underlying these common urologic diseases.

Author disclosure statement

The authors declare that they have no competing financial interests.

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